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Synergistic Effect of Apple Extracts and Quercetin 3-β-D-Glucoside Combination on Antiproliferative Activity in MCF-7 Human Breast Cancer Cells in Vitro

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Breast cancer is the most frequently diagnosed cancer in women. An alternative strategy to reduce the risk of cancer is through dietary modification. Although phytochemicals naturally occur as complex mixtures, little information is available regarding possible additive, synergistic, or antagonistic interactions among compounds. The antiproliferative activity of apple extracts and quercetin 3-β-D-glucoside (Q3G) was assessed by measurement of the inhibition of MCF-7 human breast cancer cell proliferation. Cell cytotoxicity was determined by the methylene blue assay. The two-way combination of apple plus Q3G was conducted. In this two-way combination, the EC_{50} values of apple extracts and Q3G were 2- and 4-fold lower, respectively, than those of apple extracts and Q3G alone. The combination index (CI) values at 50 and 95% inhibition rates were 0.76 ± 0.16 and 0.42 ± 0.10, respectively. The dose-reduction index (DRI) values of the apple extracts and Q3G to achieve a 50% inhibition effect were reduced by 2.03 ± 0.55 and 4.28 ± 0.39-fold, respectively. The results suggest that the apple extracts plus Q3G combination possesses a synergistic effect in MCF-7 cell proliferation.

KEYWORDS: Synergy; phytochemicals; flavonoids; apples; quercetin 3-β-D-glucoside (Q3G); cancer; antiproliferative activity

INTRODUCTION

Breast cancer is the most frequently diagnosed cancer in women. Approximately 1 million women are estimated to be newly diagnosed with breast cancer each year worldwide. Although a great deal of work has been done in the prevention and treatment of breast cancer, the results are not satisfactory and need to be greatly improved. For example, one drug, Tamoxifen, has been demonstrated to be effective in only one-third of breast cancer patients (*1*). Therefore, exploring new approaches in the prevention and treatment of breast cancer is of great interest.

One of the alternative strategies to reduce the risk of cancer is through dietary modification. It has been estimated that a healthy diet could prevent approximately 30% of all cancers (*2*). Numerous epidemiological and animal studies revealed what appeared to be a strong link between intake of fruits and vegetables and protection against cancer (*3*−*6*). Apples are widely and commonly consumed and are one of the main contributors of phytochemicals in the human diet. In the United States, apples are attributed 33% of the total phenolics consumed from fruits, making them the largest source of dietary phenolics (*7*). In Finland, apples and onions are major sources of dietary flavonoids, whereas in The Netherlands apples rank third behind tea and onions as primary sources of flavonoids (*8*). Apples are rich in hydroxycinnamic acids, dihydrochalcones, flavan-3-ols/procyanidins, anthocyanins, and flavonols. The sugar moieties involved in glycosylation are galactose, glucose, rhamnose, xylose, arabinose, and rutinose. Quercetin 3-glycosides, chlorogenic acid, catechin, epicatechin and their dimers, phloridzin, and cyanidin 3-glycosides are the main individual phenolics in apple. However, the amount of quercetin 3-glucoside was found to be low in apples (*9*, *10*). Apple consumption has been linked to a lowered risk of cancer, coronary heart disease, asthma and pulmonary function problems, and type II diabetes (*11*). Recently, a study focused on the investigation of apple intake and the risk of different cancers in Italy (*12*); it was found that there is a consistent inverse relationship between apple consumption and risk of various cancers such as those of the breast and prostate. Animal and in vitro studies have also exhibited that phytochemicals present in apples have strong antioxidant and antiproliferative activities, inhibit lipid oxidation in both humans and rats, and show cholesterol-lowering effects (*11*, *13*, *14*). Our group reported that, corresponding to doses in human consumption of one, three, and six apples a day, the whole apple extracts prevent mammary cancer in rats in a dose-dependent manner (*15*).

A synergistic therapeutic effect is defined as a stronger effect by the combination of two or more compounds compared to individual compounds as equal concentrations. It is believed that chemotherapeutic combination approaches have been used to reduce drug toxicity, to delay the development of cancer cells, and to reach a greater effect than with one active drug alone. Antioxidant synergism has been observed with different compounds such as vitamins E and C (*16*), vitamin E and β-carotene (*17*), catechin and malvidin 3-glucoside (*18*), flavonoids and urate (*19*), and tea polyphenols and vitamin E (*20*).

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In addition to antioxidant activities, phenolics have demonstrated other biological activities, interfering with cellular mechanisms. For example, Conte et al. (*21*) reported synergistic protection of PC12 cells from β-amyloid toxicity by reservatrol and catechin.

The phytochemicals in fruits may act independently or in combination as anticancer agents. The additive and synergistic effects of phytochemicals in fruits may be responsible for their potent antioxidant and anticancer activities, and the benefit of a diet rich in fruits is attributed to the complex mixture of phytochemicals present in whole foods (*22*, *23*). This hypothesis partially explains why a single antioxidant cannot replace the combination of natural phytochemicals in fruits in achieving health benefits. Limited knowledge is available regarding any interactions between/among phytochemicals in suppressing MCF-7 cell proliferation. There is no direct evidence linked to synergistic, additive, or antagonistic effects on the inhibition of MCF-7 cell proliferation by apples. To test the hypothesis that the combination of phytochemicals is responsible for the health benefit in apples, a two-way combination of apple extracts plus Q3G was designed. The objective for this study was to determine whether the apple extracts in combination with Q3G have additive and/or synergistic effects on MCF-7 human breast cancer cell proliferation.

MATERIALS AND METHODS

Chemicals. 3-(4,5-Dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfenyl)-2*H*-tetrazolium (MTS), quercetin 3-β-D-glucoside (Q3G), and other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO). Dimethyl sulfoxide (DMSO) was purchased from Fisher Scientific (Fair Lawn, NJ). All chemicals used in this study were of analytical grade.

Materials. Red Delicious apples were obtained from Cornell Orchard (Cornell University, Ithaca, NY). Three separate batches of apples collected were used to prepare triplicate samples. Fruits were cleaned and dried before extraction of phytochemicals. All data collected were reported as means ± SD for at least three replications.

Sample Extraction. Phytochemicals from fresh apples were extracted according to the modified method reported previously in our laboratory (*24*). Briefly, a whole apple was sliced using a Leifheit Proline apple corer. One hundred grams of apple slices with 100 g of 80% acetone (1:2 w/v) was added into a Waring blender. The blender speed was adjusted to medium to break the skin and pulp. Then, another 100 g of 80% acetone was added into blender, and the mixture was blended for 5 min on high speed. The mixture was then homogenized in a Virtis High Speed Homogenizer (VirTis Co., Gardiner, NY) for 5 min and filtered with a vacuum through a no. 2 Whatman filter paper in an ice bath. Solvent in the filtrate was evaporated using a rotary evaporator at 45 °C until the weight of the evaporated filtrate was < 10% of the weight of the original filtrate. The sample was then recovered with water to a final volume of 50 mL and stored at −40 °C until use. All extractions were performed in triplicate.

Cytotoxicity Measurement. Cell cytotoxicity was assessed by using the methylene blue assay (*25*, *26*). The rationale here is that the maximum combined concentration of apple extracts plus Q3G could be delivered, but without any cytotoxicity. Briefly, MCF-7 human breast cancer cells (American Type Culture Collection, ATCC, Rockville, MD) were seeded at 4×10^4 cells/well into a 96-well plate and incubated for 24 h. The cells were then treated with different concentrations of apple extracts (10, 20, 30, 40, 50, 60, 75, 100, and 125 mg/mL), Q3G (10, 20, 30, 40, 50, and 60 μM), apple extracts plus Q3G (8.75 mg/mL + 2.9 μM, 17.5 mg/mL + 5.8 μM, 35.0 mg/mL + 11.6 μM, 52.5 mg/mL + 17.4 μM, 70.0 mg/mL + 23.2 μM, and 90.0 mg/mL + 45.0 μM), or control for 24 h, respectively. For experimental analysis, the growth medium was removed, and the wells were rinsed with PBS. Fifty microliters of Hanks Balanced Salt Solution (HBSS: 1.25% glutaraldehyde and 0.6% methylene blue) was added into each well to fix and stain the cells for 1 h. The 96-well plate was rinsed several times by immersion in Milli-Q water after removal of HBSS. At the end of treatment, 100 μL of elution solution (PBS plus 50% of ethanol and 1% of acetic acid) was added, and cells were incubated for 0.5 h with gentle rotation at room temperature. Absorbance was read at 570 nm using a MRX Microplate Reader (Dynex Technologies, Chantilly, VA).

Inhibition of MCF-7 Cell Proliferation. The antiproliferative activity of apple extracts and Q3G toward MCF-7 breast cancer cells was assessed by the colorimetric MTS assay (MTS-based cell titer 96 nonradioactivity cell proliferation assay) (Promega, Madison, WI) reported previously (*27*, *28*). Briefly, MCF-7 cells were maintained in Alpha Minimum Essential Medium (MEM-α), containing 10 mM Hepes, 0.01 mg/mL insulin, 50 units/mL penicillin, 50 μg/mL streptomycin, 100 μg/mL gentamicin, and 10% fetal bovine serum (Gibco, Life Technologies, Grand Island, NY). MCF-7 cells were maintained in a 5% CO_2/37 °C incubator. MCF-7 cells were seeded in 96-well flat-bottom plates at 2.5×10^4 cells. Proliferation was measured by the ability of viable cells to reduce MTS to formazan. After 4 h of incubation, the growth medium was removed and media containing various concentrations of apple extracts (10, 30, 50, 75, 100, and 125 mg/mL), Q3G (10, 20, 30, 40, 50, and 60 μM), apple extracts plus Q3G (8.75 mg/mL + 2.9 μM, 17.5 mg/mL + 5.8 μM, 35.0 mg/mL + 11.6 μM, 52.5 mg/mL + 17.4 μM, 70.0 mg/mL + 23.2 μM, and 87.5 mg/mL + 29.0 μM), or control were added to the cells. After 96 h, cell proliferation was determined using the MTS assay measuring absorbance at 490 nm. Antiproliferation was measured as percent compared to control, and all measurements were conducted in triplicate. The effective median dose (EC_{50}) was determined and expressed as milligrams of apples per milliliter ± SD or micromolarity of Q3G ± SD.

Experimental Design of Combination Study. A two-way combination of apple extracts plus Q3G toward MCF-7 cell proliferation was designed. The EC_{50} values of apple extracts and Q3G were determined on the basis of the dose−response curve. According to each EC_{50} value, a series of concentrations at different ratios of EC_{50} were tested. For apple extracts, the combined concentrations were $0.125 \times EC_{50}$, $0.25 \times EC_{50}$, $0.5 \times EC_{50}$, $0.75 \times EC_{50}$, $1.0 \times EC_{50}$, and $1.25 \times EC_{50}$. For Q3G, the combined concentrations were $0.125 \times (EC_{50})/2$, $0.25 \times (EC_{50})/2$, $0.5 \times (EC_{50})/2$, $0.75 \times (EC_{50})/2$, $1.0 \times (EC_{50})/2$, and $1.25 \times (EC_{50})/2$. Finally, a series of concentrations of apple extracts and Q3G were mixed to generate the dose−response curve in the MCF-7 cell proliferation model. The combination effects were analyzed by the combination index (CI) as described below.

Median−Effect Principle for Dose−Effect Analysis. The dose−effect analysis was modified from that of Chou and Talalay (*29*). The median−effect principle was used to calculate individual and combined apple extracts and Q3G effects. Dose−effect curves for apple extracts and Q3G and their combinations with series diluted concentrations were plotted by using the median−effect equation

$$f_a = 1/[1 + (D_m/D)^m] \quad (1)$$

where D is the dose, D_m is the dose required for 50% inhibition effect, which is equivalent to median effect dose (EC_{50}), f_a is the fraction affected by dose D, and m is a coefficient of the sigmoidicity of the dose−effect curve.

The medium−effect plot is based on the logarithmic form of Chou's median−effect equation (*29*):

$$\log(f_a/f_u) = m \log(D) - m \log(D_m) \quad (2)$$

where f_u is the fraction unaffected, $f_u = 1 - f_a$.

Combination Index for Determining Addition, Synergism, and Antagonism. The CI based on the classic isobologram equation (*30*) has been used for data analysis of two-way combination as

$$CI = \frac{(D)_1}{(D_x)_1} + \frac{(D)_2}{(D_x)_2} \quad (3)$$

where $(D)_1$ and $(D)_2$ are the doses of apple extracts and Q3G, respectively, in the combination system; $(D_x)_1$ and $(D_x)_2$ are the doses of apple and Q3G alone, respectively. For data analysis of combinations, CI < 1, CI = 1, and CI > 1 indicate synergistic, additive, or antagonistic effects, respectively (*29*, *30*).

Statistical Analysis. Statistical analysis was performed using Minitab Student Release 12 (Minitab Inc., State College, PA) and SigmaStat Version 8.0 (Jandel Corp., San Raphael, CA). Results were subjected to

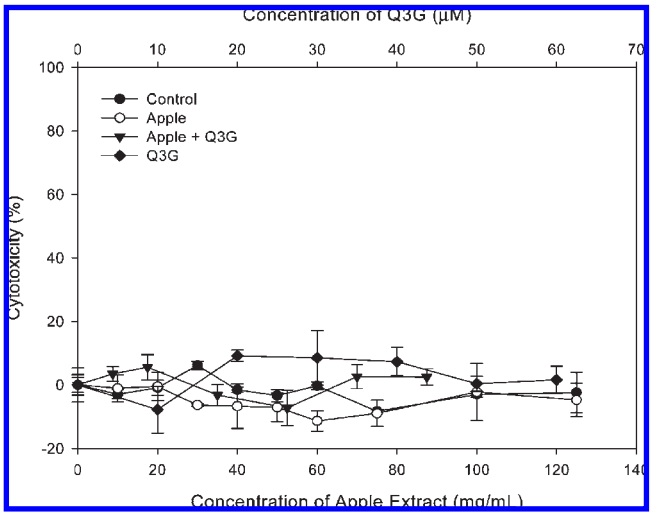

Figure 1. Effect of apple extracts, Q3G, and their combination on cytotoxicity of MCF-7 breast cancer cells.

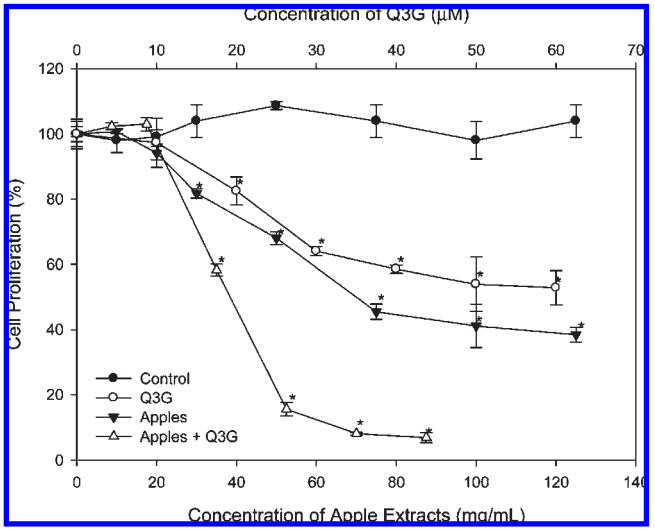

Figure 2. Synergistic interactions between apple extracts and Q3G in inhibition of MCF-7 breast cancer cell proliferation (mean ± SD, $n = 3$). ∗ indicates a significant difference from the control ($p < 0.05$).

ANOVA, and differences between means were located using Tukey's multiple-comparison test. Significance was determined at $p < 0.05$. All data were reported as the mean ± SD of three replications.

RESULTS AND DISCUSSION

Cytotoxicity. The cytotoxicity of apple extracts, Q3G, and two-way combination of those two agents (apple extracts + Q3G) toward the growth of MCF-7 human breast cancer cells in vitro is shown in **Figure 1**. No cytotoxicity was observed in apple extracts at concentrations ≤125 mg/mL. There was no cytotoxicity in Q3G toward MCF cells at the maximum level of 60 μM. Also, cytotoxicity was not detected in the combination of apple extracts (90 mg/mL) plus Q3G (45 μM).

Inhibition of MCF-7 Cell Proliferation. The antiproliferative activities of apple extracts, Q3G, and two-way combination of apple extracts plus Q3G toward the growth of MCF-7 human breast cancer cells in vitro are presented in **Figure 2**. Apple extracts inhibited the MCF-7 cell proliferation at doses of 30−125 mg/mL ($p < 0.05$), and the inhibition was dose-dependent. The EC_{50} value of apple extracts in inhibiting MCF-7 cell proliferation was 70.7 ± 5.7 mg/mL (**Table 1**). Q3G exhibited significant antiproliferative activity against MCF-7 cells at doses of 20−60 μM ($p < 0.05$) in a dose-dependent manner (**Figure 2**). The EC_{50} value of Q3G in inhibiting MCF-7 cell growth was 46.4 ± 1.3 μM.

Table 1. EC_{50} Values of Apple Extracts, Q3G, and Apple Extracts in Combination with Q3G in Inhibiting MCF-7 Cell Growth

component	EC_{50} value	
	single	combined
apple extracts (mg/mL)	70.7 ± 5.7	33.8 ± 2.9
Q3G (μM)	46.4 ± 1.3	10.8 ± 2.1

Two-way combination of apple extracts plus Q3G significantly increased antiproliferative activity toward the growth of MCF-7 human breast cancer cells in vitro when compared to the apple extracts and Q3G alone (**Figure 2**). The EC_{50} values of apple extracts and Q3G in the two-way combination were reduced to 33.8 ± 2.9 mg/mL and 10.8 ± 2.1 μM, respectively (**Table 1**), which were 2- and 4-fold lower than those of apple extracts and Q3G alone. No cytotoxicity was observed in the two-way combination of apple extracts and Q3G at all concentrations tested in MCF-7 cells.

Median−Effect Plot for Apple Extracts and Q3G Combination. The combination effect of apple extracts plus Q3G was expressed through CI and dose−reduction index (DRI). The CI and DRI values of this two-way combination effect were calculated on the basis of the method reported previously (*29*). The DRI is a measure of how much the dose of each component, in a synergistic combination, may be reduced at a given effect level compared with the doses for each component alone. The DRI is important in clinical situations, where dose−reduction leads to lowered toxicity toward the host while retaining the therapeutic efficacy. The DRI values at different inhibition rates in MCF-7 cell growth are presented in **Table 2**. The DRI values of apple extracts and Q3G at the 50% of inhibition of MCF-7 cell growth were 2.03 ± 0.55- and 4.28 ± 0.39-fold when compared to the values of apple extracts and Q3G alone. The DRI values of apple extracts and Q3G at the 95% of inhibition of MCF-7 cell growth were 4.32 ± 1.48- and 6.55 ± 0.66-fold when compared to the values of apple extracts and Q3G alone. The CI values at different inhibition rates in MCF-7 cell growth were calculated on the basis of the median−effect plot (**Figure 3**) and are presented in **Table 2**. The CI values of the two-way combination of apple extracts and Q3G at 50, 75, 90, and 95% inhibition of MCF-7 cell growth were 0.76 ± 0.16, 0.60 ± 0.12, 0.47 ± 0.10, and 0.42 ± 0.10, respectively, indicating that there was a strong synergistic effect at all concentrations tested.

Studies conducted by our group and others have shown that phytochemical extracts in fruits exhibit strong antioxidant and antiproliferative activities and that the major part of total antioxidant activity is from the combination of phytochemicals (*31*). Although there are many therapeutic strategies including chemotherapy that treat clinical breast cancer, the results have been unsatisfactory. Therefore, there is an urgent need to develop alternative strategies to prevent and treat breast cancer. Dietary modification is a practical approach that could combine relatively nontoxic fruit and vegetable phytochemicals and may enhance the efficacy of chemotherapy with a lower toxicity to normal cells. The interaction among natural phytochemicals in fruits that may play a role in antiproliferation of cancer cells is not fully understood. On the basis of the study of cytotoxicity and cell proliferation of apple extracts and Q3G alone, the two-way combination of apple extracts plus Q3G was tested in MCF-7 cells. Our data clearly exhibited that apple extracts in combination with Q3G had a potent synergistic effect toward MCF-7 cell proliferation in vitro.

Substantial observational evidence regarding the potential role of antioxidant nutrients in the prevention of cancers has been

Table 2. CI and DRI Values for Apple Extracts and Q3G Combination at 50, 75, 90, and 95% Inhibition of MCF-7 Cell Growth

	DRI[a] values at inhibition of				CI values at inhibition of			
component	50%	75%	90%	95%	50%	75%	90%	95%
apple extracts	2.03 ± 0.55	2.69 ± 0.79	3.56 ± 1.15	4.32 ± 1.48	0.76 ± 0.16	0.60 ± 0.12	0.47 ± 0.10	0.42 ± 0.10
Q3G	4.28 ± 0.39	5.00 ± 0.22	5.87 ± 0.06	6.55 ± 0.66				

[a] DRI (dose−reduction index) represents the order of magnitude (fold) of dose reduction that was allowed in combination for a given degree of effect as compared with the dose of each component alone. All DRI values were calculated on the basis of the classic isobologram equation and assumptions (*30*).

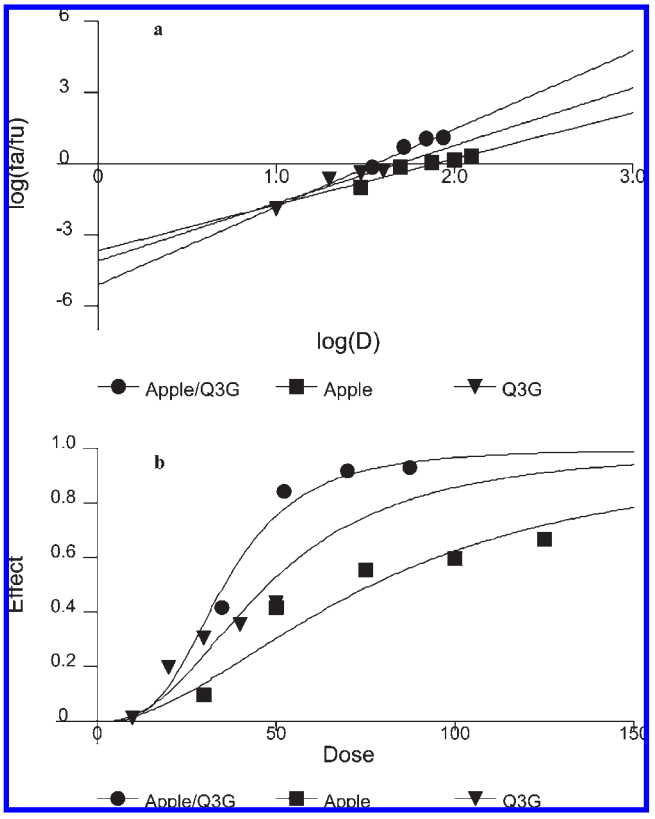

Figure 3. Median−effect (**a**) and dose−effect (**b**) plots for interactions between apple extracts and Q3G combination.

greatly increased over the past few decades. However, in some clinical trials, it is noted that antioxidant nutrients taken alone do not explain the observed health benefits of diets rich in fruits and vegetables (*23*). For example, in a randomized, double-blind, and placebo-controlled β-carotene trial, 22071 male physicians that had supplemented their diets with β-carotene at 50 mg every other day, or placebo, showed no differences in malignant neoplasms, CVD, or death from all causes (*32*). A major question still exists of why the single-antioxidant approach does not work in clinical trials. The crucial point is finding out if a purified phytochemical has the same health benefit as does the whole mixture of foods in which the phytochemicals are present (*23*). It is hypothesized that the additive and synergistic effects of phytochemicals in fruits and vegetables are responsible for their potent antioxidant and anticancer activities and that the benefit of a diet rich in fruits and vegetables is attributed to the complex mixture of phytochemicals present in whole foods (*23*).

It is estimated that approximately 8000 phytochemicals are present in whole foods (*23*), and there are quite possibly many more. Those compounds with much more complex scope, interaction, and magnitude may act on different targets with different mechanisms of action. It is believed that phenolics can exert their effects on the different signaling pathways such as mitogen-activated protein kinases (MAPK), activator protein-1 (AP-1), or nuclear factor-κB (NF-κB) either separately or sequentially, as well as possibly interacting between/among these pathways, which can offer complementary and overlapping mechanisms of action. Apple extracts have been demonstrated to have the capabilities of inhibiting NF-κB activation in MCF-7 cells (*33*). The effects of 2α-hydroxyursolic acid isolated from apple peels on cell proliferation and TNF-α-induced NF-κB activation were investigated (*34*). Pre-incubation of MCF-7 cells with 2α-hydroxyursolic acid suppressed TNF-α-induced NF-κB activation. 2α-Hydroxyursolic acid treatment did not have an effect on the phosphorylation level of NF-κB inhibitor (IκB-R), but proteasome activity was inhibited at doses of 10 and 20 μM ($p < 0.05$), indicating that 2α-hydroxyursolic acid has antiproliferative activities against MCF-7 cells and capabilities inhibiting NF-κB activation induced by TNF-α partially by suppressing proteasome activities (*34*). Some of the extensively studied bioactive compounds in apples include catechin, epicatechin, procyanidin, cyanidin-3-galactoside, coumaric acid, chlorogenic acid, gallic acid, quercetin 3-galactoside, quercetin 3-glucoside, quercetin 3-rhamnoside, and phloridzin. Apples contain high amounts of phenolics and flavonoids (*35*−*37*). The free phenolic content in apple extracts was 272.1 ± 6.2 mg/100 g (*35*). Tsao et al. (*10*) examined the concentrations of the major phenolic compounds in eight apple cultivars. The total phenolics ranged from 101.7 to 235.0 mg/100 g of fresh weight. Procyanidins were present in both the peel (59.7%) and the flesh (55.7%). Ranging from 22.03 to 34.99 mg/100 g in the peels of the eight varieties, quercetin glycosides accounted for 17.9% of the total phenolics; they were primarily composed of quercetin 3-galactoside, 3-arabinoside, and 3-rhamnoside. The amount of Q3G present in apple was found to be low (*9*, *10*, *36*), which may be not enough to have antiproliferative activity against MCF-7 cells. Recently, 42 bioactive constituents in Red Delicious apple peels, including triterpenoids, flavonoids, organic acids, and plant sterols, were isolated and further identified utilizing HR-MS and 1D and 2D NMR (*36*, *37*). It was found that the main flavonoids in apple peels are quercetin 3-*O*-β-D-glucopyranoside and quercetin 3-*O*-β-D-galactopyranoside, followed by minor amounts of quercetin, (−)-catechin, (−)-epicatechin, and quercetin 3-*O*-*R*-L-arabinofuranoside. Among the compounds isolated, quercetin and quercetin 3-*O*-β-D-glucopyranoside exhibited potent antiproliferative activities against MCF-7 cells (*36*). The results in this study showed that the two-way combination of apple extracts and Q3G had strong synergistic action against MCF-7 cell growth, which may be of interest in clinical trials for breast carcinoma.

Bioactive compounds can offer additive or synergistic interaction through different biochemical targets. For example, quercetin could enhance the action of carboxyamidotriazole (CAD) in human breast carcinoma MDA-MB-435 cells (*38*). When quercetin and CAD were added to the MDA-MB-435 cells, synergism was observed in isobolograms in growth inhibition and clonogenic assays. The most effective combinationd wrtr 20 μM quercetin with 4 μM CAD in growth inhibition assay and 30 μM quercetin with 1.2 μM CAD in clonogenic assay. In combination, resveratrol and quercetin activated caspase 3 in human pancreatic carcinoma cells and synergistically induced

apoptosis in human leukemia cells (*39*). The effect of a combination of quercetin (25 μM) and *trans*-resveratrol (25 μM) on mitochondrial cytochrome *c* release and caspase 3 activity was greater than the expected additive response. The synergistic action with those two agents supports the concept that the flavonoids (quercetin) and nonflavonoids (*trans*-resveratrol) act on the membrane permeability transition by distinct pathways, suggesting an interaction between the two. Synergistic interactions with a CI of 0.64 for the combination of ellagic acid and resveratrol and a CI of 0.68 for quercetin and resveratrol were observed after an isobolographic analysis, indicating that the anticarcinogenic potential of foods containing phenolics may not be based on the effects of individual compounds but may involve a synergistic enhancement of the anticancer effects (*40*). In this study, the combined concentration was almost saturated in the dose−effect curve, which did not show cytotoxicity in the maximum combined concentration. The dose−response curve of antiproliferative activity was shifted to the left after combination of apple extracts and Q3G (**Figure 3b**). The EC_{50} value of apple extracts and Q3G after combination is lower than the EC_{50} of apple extracts or Q3G alone, suggesting synergistic effect. In addition, the CI at 50% inhibition rate in apple extracts and Q3G combination was < 1, indicating a synergistic action (*29*, *30*). The mechanism of action in apple extracts plus Q3G combination might be explained by the theory that bioactive compounds in apple extracts tend to increase the antiproliferative activity in MCF-7 cells by suppressing one or more targets of the signal transduction pathway, by stabilizing the Q3G in the system, or by increasing the bioavailability of the Q3G (*41*).

The health benefits of fruits and vegetables are likely due to the additive and synergistic effects of an array of phytochemicals, rather than to a single compound alone. The reasons may be due partially to the loss of purified compounds, the loss of their bioactivity, or the possibility that they may not behave the same as in whole food (*23*). In addition, these compounds differ in molecular size, polarity, and solubility, which may affect their bioavailability and distribution in different subcellular organelles, cells, tissues, and organs. Other explanations of the contradictory results between observational studies and randomized trials could be the fact that the doses used in clinical trials have been much higher than what is normally consumed in foods. Consumers may gain more significant health benefits from whole foods in their balanced diet than from dietary supplements, which do not contain the same array of complex compounds (*23*). This study demonstrates a synergistic effect of apple extracts and Q3G in MCF-7 cell proliferation model. Our findings have important implications for combinations of phenolics in the prevention of cancer. However, future studies are needed to elucidate the underlying mechanisms of combination effects of bioactive components in breast cancer. Moreover, further mechanistic and human clinical studies may lead to novel approaches in the prevention of cancer from a dietary source for enhancing efficacy while lowering cytotoxicity to normal cells.

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