The Cytotoxic and Synergistic Effects of Flavonoid Derivatives on Doxorubicin Cytotoxicity in Hela, MDA-MB-231, and HT-29 Cancer Cells

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ABSTRACT

Background: Flavonoids have a variety of biological activities, such as anti-allergic, anti-inflammatory, anti-oxidative, free radical scavenging, and anti-mutagenic.

Methods: The cytotoxic effects of three synthesized flavonoid derivatives (K3, K4 and K5) were evaluated against Hela , MDA-MB -231 and HT-29 cancer cells using MTT assay.

Results: The results showed that these flavonoids were not cytotoxic at any tested concentrations (0.1, 0.05, 0.01, and 0.001 mM). To evaluate the possible synergistic effect of synthetic flavonoids with chemotherapeutic agent, the compound (K4) was examined against Hela and MDA-MB-231 cells in combination with different concentrations of doxorubicin (0.01 and 0.001mM). This combination treatment significantly increased the cytotoxicity of doxorubicin ($P < 0.05$).

Conclusion: Synthesized flavonoids could be used either in combination therapy with other chemotherapeutic agents or used as antioxidants in food supplements.

Keywords: Hela, HT-29, MDA-MB-231, MTT Assay, Synergism, Synthetic Flavonoid.

INTRODUCTION

In the past 10 years, researchers have focused on natural products as leading compounds to find new valuable drugs to treat different diseases (1). Flavonoids are an extensive group of poly phenolic compounds existing in plants. They have been found in dietary components, including fruits, vegetables, olive oil, and tea (2). These compounds have biological activities and can be used as anti-allergic, anti-inflammatory, anti-oxidative, free radical scavenging, and anti-mutagenic activities (3). Flavonoids possess a skeleton of chromane with an additional aromatic ring attached at positions 2, 3, or 4 as seen in Figure 1 (4).

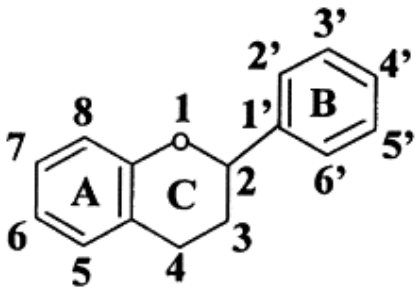

Figure 1. The common chemical structure of flavonoids

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Many studies have demonstrated that flavonoids are also potent inhibitors of key enzymes participating in signal transduction. They inhibit several kinases such as protein kinase C, tyrosine kinases, and lipid kinases (5). Moreover, they affect various metabolic pathways, such as activation of glycolytic enzymes or protein synthesis (6), and promote cell cycle arrest in G0/G1 or G2/M phase (7,8). Flavonoids also interact with estrogen type II binding sites to regulate mammary cell growth (9), and induce apoptosis in different cells lines (10). Many studies have shown that flavonoids structure which have a hydroxyl group in C3 position, such as kaempferol, quercetin, and taxifolin are associated with a 2 to 10-fold lower cytotoxicity compared to molecules that do not possess this residue, including apigenin, luteolin, and eriodictyol (11).

Multi-drug resistance (MDR) is a major problem to successful chemotherapeutic treatment in cancer patients. Among the different cellular mechanisms causing MDR, the overexpression of ATP-binding cassette (ABC) transporters represents the most common mechanism that leads to decreased effectiveness of anti-cancer drugs (12). Several flavonoids have been shown to be able to increase accumulation of anti-cancer drugs in resistant human cancer cells. Quercetin and its methoxylated derivatives inhibited the efflux of rhodamine-123 and restored sensitivity to doxorubicin in MCF-7 breast cancer cells. Quercetin was shown to bind to purified p-glicoprotein (P-gp), a plasma membrane transporter responsible for the export of chemotherapeutic agent, and efficiently inhibits its activity. It has been shown that morin, biochanin A, phloretin, and silymarin increase the accumulation of [3H]-daunomycin in P-gp over expressing MCF-7 cells (13). It was reported that quercitin can restore sensitivity to doxorubicin in multidrug-resistant cells (14). Another study using a reconstituted P-gp system showed that quercetin inhibited P-gp-mediation, at least in part by inhibiting the ATP-binding site of P-gp, in MDR positive MCF-7 cells (15).

It was revealed that in order to enhance the cytotoxic effects of flavonoids in combination with chemotherapeutic agents, presence of C2–C3 double bond is essential. The presence of this double bond leads to a planar structure of the A and C rings in the flavonoid backbone and has been linked to efficient binding and inhibition of the P-gp. Inhibition of this important cellular detoxification system may thus contribute to flavonoid-induced cytotoxicity (11).

In the present study, the cytotoxic effects of 3 synthetic flavonoid derivatives against a panel of cancer cell lines, including Hela, MDA-MB-231, and HT-29 were studied (Table 1). We also evaluated the cytotoxic effect of compound K4 in combination with doxorubicin against Hela and MDA-MB-231 cancer cells.

MATERIALS AND METHODS

Preparation of stock solutions

Appropriate amounts of synthesized compounds were dissolved in dimethylformamide (DMF) to prepare the stock solution and diluted in RPMI medium to the final concentrations of 0.1, 0.05, 0.01, and 0.001 mM. The negative control cells were treated with the same amount of vehicle alone. The final DMF concentration never exceeded 0.1% (v/v) in either control or treated samples.

Table 1. The structure and molecular weight of synthetic flavonoids used in this study

item	ID	R	MW
1	K3	$- CH_3$	330
2	K4	$CH_3 - CH_2 -$	344
3	K5	$CH_2 = CH- CH_2-$	356

Materials

The tested compounds were already synthesized at the Department of Pharmaceutical Chemistry, School of Pharmacy, Shaheed Beheshti University, Tehran, Iran.

DMEM and RPMI 1640 media (GIBCO, USA), fetal bovine serum (FBS; GIBCO, USA.), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma, USA), and Hoechst33258 and propidium iodide (PI; Sigma, USA) were purchased from local vendors. Other chemicals were of analytical grade and were provided from commercial suppliers.

Cell lines and culture conditions

The human cancer cell lines used in this study included MDA-MB-231, Hela and HT-29 were purchased from the cell bank of Pasteur Institute, Tehran, Iran. The cells were cultured in RPMI 1640 (Hela and HT-29) or in DMEM (DMA-MB-231). The media was supplemented with 10% heat-inactivated bovine serum and 1% penicillin/streptomycin. All cells were incubated at 37 ◦C in a humidified atmosphere of 95% air and 5% CO2 and subcultured when the cells had 85% confluence.

Cytotoxicity assay

MTT colorimetric assay was performed as described before (16). MDA-MB-231, Hela and HT-29 cells were placed in 96-well culture plates (10000 cells/well) and allowed to attach for 24 hours before treatment. Then they were treated with different concentrations of synthetic flavonoids (K3, K4 and K5) for 72 hours. Absorbance of viable cells was measured at 540 nm using ELISA plate reader. Percent cell survival was calculated compared to the untreated cell (negative control) that was assumed as 100% viable.

RESULTS

Effects of synthetic flavonoids against HT-29, Hela, and MDA-MB-231 cells

To determine the sensitivity of cells to the cytotoxic/cytostatic effects of 3 synthetic flavonoids, cells were treated with various concentrations of K3, K4, and K5 as mentioned in materials and methods section. As shown in Figures 2-4, these flavonoids had no cytotoxic effects at any tested concentrations (i.e., the IC50> 100 μM) (P< 0.05).

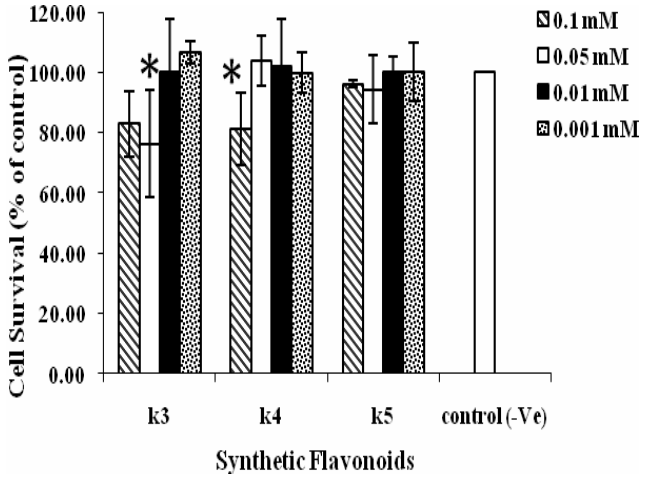

Figure 2. The cytotoxic effect of synthetic flavonoids against HT-29 cell line
* Significant difference (P< 0.05) compared to the negative control

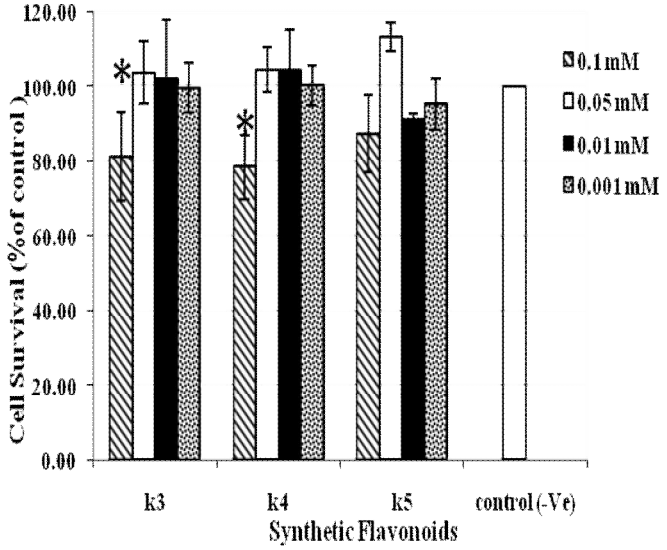

Figure 3. The cytotoxic effect of synthetic flavonoids against Hela cell line
* Significant difference ($P < 0.05$) compared to the negative control

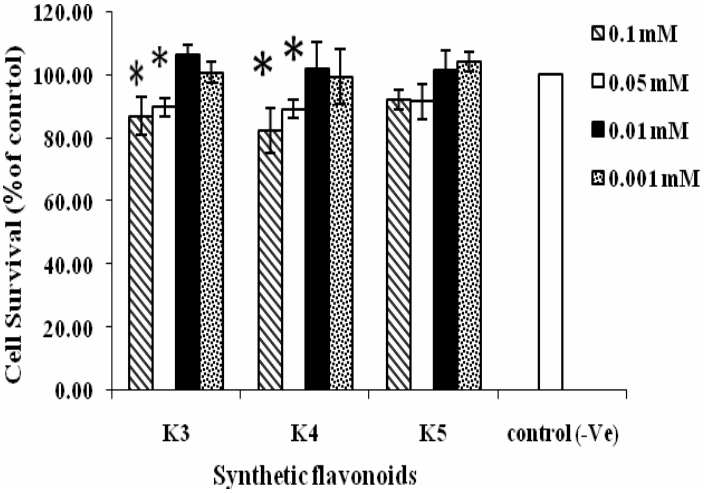

Figure 4. The cytotoxic effect of synthetic flavonoids against MDA-MB-231 cell line
*Significant difference ($P < 0.05$) compared to the negative control

Synergistic effects of combination of synthetic K4 and doxorubicin on Hela and MDA-MB-231 cells

To evaluate the possible synergistic effects of synthetic flavonoids and chemotherapeutic agent, K4 compound was tested against Hela and MDA-MB-231 cells in combination with different concentrations of doxorubicin. As shown in Figures 5 and 6, this combination treatment significantly increased

the cytotoxicity of doxorubicin ($P < 0.05$). The decreased IC 50 values are shown in Table 2.

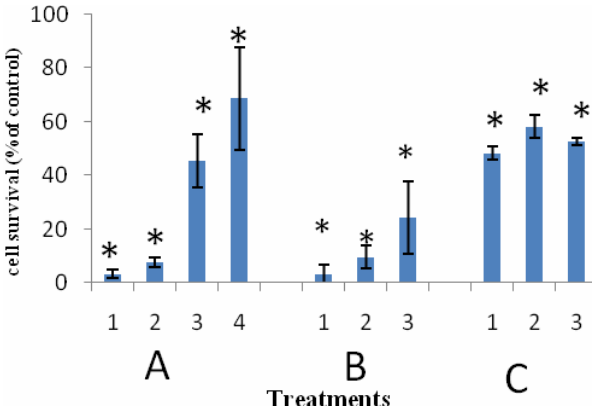

Figure 5. The cytotoxic effect of doxorubicin (A) and combination of doxorubicin and synthetic flavonoid K4 (B, C) against Hela cell line at following concentrations:
A [Dox. with concentrations of 0.1 mM (**1**), 0.05 mM (**2**), 0.01 mM (**3**), and 0.001 mM (4)]
B [Dox. (0.01mM) + different concentrations of K4: 0.1mM (**1**), 0.01mM (2), and 0.001mM (3)]
C [Dox. (0.001mM) + different concentrations of K4: 0.1mM (**1**), 0.01mM (2), and 0.001mM (3)];
* Significant difference ($P < 0.05$) compared to the negative control
Dox. = doxorubicin

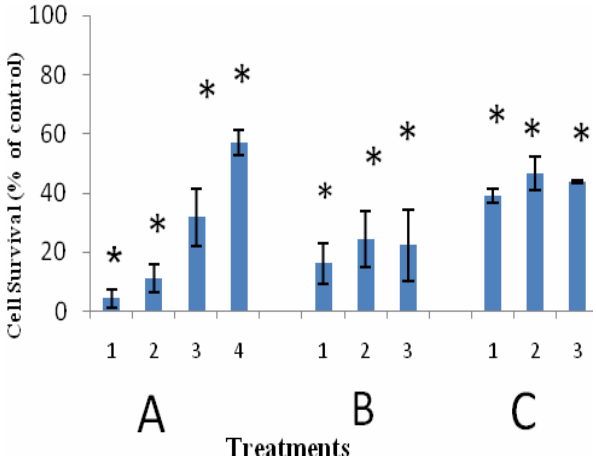

Figure 6. The cytotoxic effect of doxorubicin (A) and combination of doxorubicin and synthetic flavonoid K4 (B, C) against MDA-MB-231 cell line (see Figure 5 for the treatment concentrations)

Table 2. Percent cell survival and IC50 value of Doxorubicin, flavonoid K4, and their combination treatment in MB 231 and Hela cell lines MDA

Compound (conc.µM)	Cell survival(% of control)		* IC 50(µM)	
	MDA-MB-231	Hela	MDA-MB-231	Hela
DOX				
1	66.3 ± 4.4	68.8 ± 9.2	3 ± 0.2	3.7 ± 0.3
10	36.9 ± 9.7	45.4 ± 6.6		
Flav. (K4)				
1	101.9 ± 8.4	100.3 ± 5.3		
10	99.1 ± 8.7	104.5 ± 8.4	>100	>100
100	82.3 ± 2.8	78.51 ± 8.5		
DOX + K4 (1 to 1)				
10 + 10	28.3 ± 6.8	9.5 ± 4.2	1.1 ± 0.1	1.2 ± 0.18
1 + 1	50.9 ± 5.5	53.4 ± 0.9		
DOX + K4 (1 to 10)				
10 + 100	19.3 ± 9.32	3.2 ± 3.6		
1 + 10	54.1 ± 1.1	58.2 ± 4.3	1.3 ± 0.17	1.4 ± 0.13

*The molar drug concentrations required to cause 50% growth inhibition (IC50) were determined from dose–response curves. Results represent means ± SE of at least three different experiments.

DISCUSSION

According to Figures 2-6, the tested compounds were not cytotoxic alone against mentioned cell lines. In this regard, previous studies showed that flavonoids which possess C2-C3 unsaturated bond and a carbonyl group at position 4 exhibit lower IC50 when used in combination therapy with other chemotherapeutic agents (14). The tested compounds in this group had similar structure and, therefore, the weak observed cytotoxic activities could be related to the character of the compounds. On the other hand, compounds lacking one or both chemical features are less potent antioxidant than those possessing both functional groups (15, 17). These two functional groups may increase the activity of compounds by affording more stable flavonoid radicals via conjugation and electron delocalization. Compounds possessing an electron donating group, such as methoxy (OMe) at C3 and lipophile group at C4, were less cytotoxic (17). The tested compounds in this study had similar structure and their lower cytotoxic effect against tested cell lines may be related to their structure.

In conclusion, synthetic compounds do not have cytotoxic effects but they present synergistic effects when combined with other chemotherapeutic agents (Table 2). In addition, they might be used as antioxidants in food supplements.

ACKNOWLEDGMENTS

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