Anticancer and apoptosis-inducing effects of quercetin *in vitro* and *in vivo*

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Abstract. The present study focused on the elucidation of the putative anticancer potential of quercetin. The anticancer activity of quercetin at 10, 20, 40, 80 and 120 μM was assessed *in vitro* by MMT assay in 9 tumor cell lines (colon carcinoma CT-26 cells, prostate adenocarcinoma LNCaP cells, human prostate PC3 cells, pheocromocytoma PC12 cells, estrogen receptor-positive breast cancer MCF-7 cells, acute lymphoblastic leukemia MOLT-4 T-cells, human myeloma U266B1 cells, human lymphoid Raji cells and ovarian cancer CHO cells). Quercetin was found to induce the apoptosis of all the tested cancer cell lines at the utilized concentrations. Moreover, quercetin significantly induced the apoptosis of the CT-26, LNCaP, MOLT-4 and Raji cell lines, as compared to control group ($P<0.001$), as demonstrated by Annexin V/PI staining. In *in vivo* experiments, mice bearing MCF-7 and CT-26 tumors exhibited a significant reduction in tumor volume in the quercetin-treated group as compared to the control group ($P<0.001$). Taken together, quercetin, a naturally occurring compound, exhibits anticancer properties both *in vivo* and *in vitro*.

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Introduction

The development or identification of compounds capable of killing transformed or cancer cells, without being toxic to their normal counterparts, is of utmost importance, and has gained the increasing interest of scientists worldwide. Since antiquity, plants have been considered rich sources of chemicals, with immense therapeutic potential. During recent years, some of these plant-derived compounds or phytochemicals have been shown to be highly competent anticancer agents, in addition to being effective against many other diseases (1-4).

Cancer, following cardiovascular diseases, is the main cause of mortality and morbidity in Europe. The key characteristics of this aggressive disease are uncontrolled growth and the spread of transformed cells (5). Each year, millions of individuals are diagnosed with cancer, whereas approximately 3.5 million cancer-related deaths are annually recorded worldwide (6,7). Specifically, only in Europe, approximately 3.45 million new cases of cancer were reported in 2012, excluding non-melanoma skin cancer, whereas approximately 1.75 million deaths occurred (8).

Throughout history, plant extracts and their purified active components have been the backbone of cancer chemotherapeutics (9). It is estimated that over 70% of anticancer compounds are either natural products, or natural product-derived substances (10).

Natural polyphenols are a large and abundant group of phytochemicals found in herbal beverages and food (11-13). The plethora of these compounds is highlighted by the fact that up to date, over 8,000 polyphenols have been identified.

Structurally, polyphenols consist of aromatic rings which are functionalized with one or more hydroxyl groups (12). Flavonoids and phenolics in particular, represent an important component of a normal human diet (14,15). The average daily flavonoid intake varies from approximately 1-2 g per day (16), depending on the type and the amount of fruit, vegetables or beverages consumed. The existence of a negative correlation between a diet rich in polyphenols and various diseases, such as cancer, cardiovascular and degenerative diseases has been well established (4,17). The total phenolic content (TPC) and the total flavonoid content (TFC) seem to correlate positively with *in vivo* and *in vitro* anticancer and antioxidant properties (18). Indeed, The American Institute of Cancer Research has reported that a high intake of fruit and vegetables correlates with a low risk of tumor occurrence (19). In addition, The National Academy of Sciences of the United States (1982) has also laid stress on the importance of fruits and vegetables in cancer prevention by including respective guidelines in its report on diet and cancer (20-22). The polyphenolic compounds have been reported to have many pharmacological activities, such as antioxidant, anti-inflammatory, anticarcinogenic, antiviral, or antiallergic effects (3,4,23-25).

Among anticancer and cancer preventing drugs, flavonoids are the most studied ones. These compounds can interfere with specific stages of the carcinogenic process, inhibit cell proliferation and induce apoptosis in several types of cancer cells (26-30). Flavonoids demonstrate a notable antioxidant activity, not only by inhibiting ROS generation, but also by affecting the activity of sundry detoxifying enzymes, such as cyclooxygenases, lipoxygenases and inducible nitric oxide synthase (3,31-33). This antioxidant capacity of flavonoids could possibly account for their anticancer potency. Flavonoids have also been found to influence epigenetic alterations by chromatin remodeling (34,35).

Quercetin (3,3',4',5,7-pentahydroxyflavone) belongs to polyphenolic flavonoids which are abundantly found in apples, red grapes, onions, raspberries, honey, cherries, citrus fruits and green leafy vegetables, and exerts various biological effects, including antioxidant, anticancer, antiviral, apoptosis-inducing, protein kinase C-inhibitory, cell cycle modulatory and angiogenesis inhibitory effects. Indeed, quercetin is a unique compound due to its potential to combat cancer-related diseases in a multi-targeted manner (36-38). A number of studies have investigated the anticancer activity of quercetin (39-41). In particular, it has been reported that quercetin at various concentrations, suppresses tumor growth of various cancer cell lines, including breast, colorectal, stomach, head and neck, lung, ovarian, melanoma and leukemia (42-50). In addition, quercetin has been shown to inhibit the release of P-glycoprotein in the MCF-7 cell line and to enhance *in vitro* anticancer activity of adriamycin in breast cancer cell line (44).

Furthermore, it has been suggested that the chronic administration and daily intake of quercetin may be useful for prevention of some cancer types (51). However, cancer treatment in many cases is not effective, with disease recurrence and/or spreading leading to poor outcomes (52). Thus, development of novel therapeutic strategies remains an important goal in the on-going battle against cancer.

The aim of this study was to investigate the anticancer effects of quercetin *in vitro* and *in vivo*. The *in vitro* experiments were performed using a cohort of 9 representative cell lines. We examined the effects of quercetin on cancer cell proliferation (using MTT assay) and apoptosis [utilizing flow cytometer Annexin V/propidium iodide (PI) and TUNEL assay]. In mice bearing MCF-7 and CT-26 xenografts, we also performed an *in vivo* evaluation of the effects of quercetin.

Materials and methods

Cell culture. We used the following cancer cell lines: CT-26 which is a mouse colon carcinoma cell line that is widely used for drug development (53); PC-12 cell line which is derived from pheocromocytoma and has been widely used as a model of neuronal differentiation (54); LNCaP (androgen-sensitive cancer line) and PC-3 (androgen-insensitive cancer line) utilized as prostate cancer models; MOLT-4 [the T-cell line that causes acute lymphoblastic leukemia (ALL)], U266B1 (human myeloma cell line) and the Raji cell line (derived from human lymphoid) utilized as blood cancer models; we also used MCF-7 cells (estrogen receptor-positive breast cancer cells) as a breast cancer cell model; and CHO (ovarian cancer cell line) utilized to study ovarian cancer responses. The cancer cell lines (Pasteur Institute, Tehran, Iran) were grown under the following conditions: the CT-26, MOLT-4, U266B and PC12 in RPMI-1640 (Sigma, St. Louis, MO, USA); the PC3 cells in Ham's F12 (Sigma) and the LNCaP, MCF-7, Raji and CHO cells in DMEM (Sigma) supplied with 10% FBS (Gibco, Grand Island, NY, USA) and penicillin-streptomycin (Sigma) at 37˚C in a humidified incubator containing 5% CO_2. All experiments were performed when the cells reached 85-90% confluence.

Measurement of cell viability by MTT assay. Dilution series (10, 20, 40, 80 and 120 μM) of quercetin were prepared and used for 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. All 9 cancer cell lines were seeded at a density of $1.5x10^4$ cells/well and treated with a range of concentrations in triplicate in 96-well cell culture plates, whereupon cell proliferation was assessed using a standard MTT assay. Specifically, the growth inhibitory activity of quercetin was determined using MTT, which correlates the cell number with the mitochondrial reduction of MTT to a blue formazan precipitate. In brief, the cells were plated in 96-well plates and allowed to attach overnight. The medium was then replaced with serum-free medium containing the test compounds and cells were incubated at 37˚C for 72 h. The medium was then replaced with fresh medium containing 1 mg/ml MTT. Following incubation at 37˚C for 2-4 h, the wells were aspirated, the dye was solubilized in DMSO and the absorbance was measured at 570 nm using a BioTek Instruments EL800 Microplate Reader (BioTek Instruments, Inc., Winooski, VT, USA). The viability of cells was compared with that of the control cells.

Detection of necrosis and apoptosis by flow cytometry. For the evaluation of apoptosis using the Annexin V/PI method, the LNCaP, CT-26, MOLT-4 and Raji cells were seeded in 12-well plates ($2x10^5$ cells/well). The cells were cultured and incubated (with 5% CO_2 and 95% air) at 37˚C. Various concentrations of quercetin (10, 20, 40, 80 and 120 μM were dissolved

in DMSO and incubated with the cells for 48 h. DMSO in culture medium never exceeded 0.1% (v/v), the concentration known not to affect cell proliferation. The Annexin V-FITC/PI apoptosis kit (Abcam, Cambridge, MA, USA) was used. For this purpose, the cells were incubated with 5 μl Annexin V-FITC and 5 μl PI for 5 min in the dark. The treated cells were analyzed using a Partec PAS flow cytometer (Sysmex Partec GmbH, Gorlitz, Germany).

In vivo experiments. Female BALB/c mice, aged 6-8 weeks (weighing, 20-25 g) were obtained from Zabol University of Medical Sciences (Zabol, Iran). The animals were maintained in a temperature and humidity-controlled room. On day 1, the animals were shaved on the back flank. In the shaved right flank of each mouse, $3x10^5$ CT-26 or MCF-7 cells in 50 μl PBS were injected subcutaneously, as previously described (55). Eight days after tumor implantation, the animals were randomly assigned into 8 groups as follows (5 mice in each group): i) CT-26 tumor-bearing mice treated with dextrose 5% (CT-26 control group); ii) CT-26 tumor-bearing mice treated with 50 mg/kg [intraperitoneally (i.p.)] quercetin; iii) CT-26 tumor-bearing mice treated with 100 mg/kg (i.p.) quercetin; iv) CT-26 tumor-bearing mice treated with 200 mg/kg (i.p.) quercetin; v) MCF-7 tumor-bearing mice treated with dextrose 5% (MCF-7 control group); vi) MCF-7 tumor-bearing mice treated with 50 mg/kg (i.p.) quercetin; vii) MCF-7 tumor-bearing mice treated with 100 mg/kg (i.p.) quercetin; and viii) MCF-7 tumor-bearing mice treated with 200 mg/kg (i.p.) quercetin. The tumor volume was measured and calculated based on the following formula: [(length) x(width)2]/2, as previously described (56,57).

In addition, the animal survival rate was evaluated up to 40 days. Furthermore, for apoptosis evaluation in the animals, terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) assay was performed. The study protocol was approved by the Ethics Committee of Zabol University of Medical Sciences. All experimental procedures conformed to the declaration of Helsinki and were conducted in accordance with recent legislation of National Institutes of Health guide for the care and use of laboratory animals.

In situ apoptosis assay. Implanted tumor tissues were collected and apoptosis was detected using TUNEL assay. Based on the instructions of the manufacturer (Roche Diagnostics, Basel, Switzerland), paraformaldehyde-fixed blocks were embedded in paraffin, cut into 4-μm thick slices, and incubated with TUNEL reaction mixture containing TdT and fluorescein-dUTP. Prior to incubation of the slices with TUNEL mixture, their permeability was enhanced by proteinase solution. The TUNEL signal was then detected by an anti-fluorescein antibody conjugated with alkaline phosphatase (*in situ* Cell Death Detection kit; 11684809910 Roche; Sigma), a reporter enzyme, which catalytically generates a colored product. Three slides from each block and four slide fields were evaluated for the percentage of apoptotic cells. Four fields were randomly selectd on each slice and the number of apoptotic myocardial cells/total myocardial cells was calculated, at x200 magnification. At the end of this procedure, the apoptotic index (number of myocardial nuclei labeled by the TUNEL method/number of total myocardial nuclei) was calculated, as previously described (58).

Statistical analysis. Statistical analysis was performed using the Student's t-test. Data are presented as the means ± standard deviation of 3 independent treatments. A value of $P<0.05$ was considered to indicate a statistically significant difference.

Results

Cell viability. MTT assay was used to evaluate the viability of all 9 cancer cell lines following 24, 48 and 72 h of treatment with quercetin at 10, 20, 40, 80 and 120 μM (Table I). All utilized concentrations of quercetin were inhibitory with the exception of the PC3 and CHO cells where at the 24 h time point, no inhibition was evident even at the highest concentration (120 μM) as presented in Table II. Furthermore, MTT assay revealed that the inhibitory effect of each concentration of quercetin on the viability of all cancer cell lines was enhanced by an increase in the incubation time. In addition, we calculated the IC_{50} values as previously described by Entezari Heravi *et al* (59). Therefore, the inhibitory effect of quercetin on the growth of utilized cancer cell lines was dose- and time-dependent, as demonstrated by the obtained IC_{50} values of quercetin (Table II). It is noteworthy, based on our cancer cell line panel (Table II), that we selected cell lines of lower and higher sensitivity to quercetin.

Determination of the apoptosis of CT-26, LNCaP, MOLT-4 and Raji cell lines following treatment with quercetin. In continuation, we examined the apoptotic rate in a panel of 4 cell lines of high and low sensitivity to quercetin (i.e., CT-26 and LNCaP, as well as MOLT-4 and Raji, respectively). Our results revealed that quercetin initiated the apoptotic process in these cells in a dose-dependent manner (Fig. 2). The effect of quercetin at 120 μM on the apoptotic rate of the CT-26, LNCaP, MOLT-4 and Raji cell lines in comparison to the control group ($P<0.001$) is depicted in Fig. 1. The results of MTT assay were compatible with the results of Annexin V/PI assay, as the cell lines with a higher sensitivity to quercetin exhibited a higher apoptotic rate ($P< 0.001$; Figs. 1 and 2).

Effect of quercetin treatment on tumor volume. In continuation, we evaluated the *in vivo* effect of quercetin on CT-26 and MCF-7 tumors; these cells lines exhibited a relatively lower sensitivity to quercetin in *in vitro* experiments (Table II). Therefore, mice bearing CT-26 and MCF-7 tumors were treated with various concentrations of quercetin (50, 100 and 200 mg/kg; i.p.) This approach revealed that the administration of quercetin significantly reduced the tumor volume at all 3 utilized doses on day 18 post-treatment for CT26 tumors and on day 20 post-treatment for MCF-7 tumors ($P<0.001$; Fig. 3).

Animal survival rate. *In vivo* experiments demonstrated that the survival rate for mice bearing CT-26 and MCF-7 tumors that were treated with the two higher concentrations of quercetin (100 and 200 mg/kg) was significantly higher compared to the control group ($P<0.05$ and $P<0.01$, respectively). No significant difference in the survival rate was evident between the group treated with quercetin at 50 mg/kg and the control group ($P>0.05$; Fig. 4).

Table I. Data obtained from MTT assay on the cell viability of different cell lines treated with quercetin (10, 20 , 40 , 80 and 120 μM) for 24, 48 and 72 h.

A, Cells treated for 24 h

Cell line	10μM	20 μM	40 μM	80 μM	120 μM
CT-26	94.2±4.4	83.5±3.7	75.1±4.2	65.8±5.5	49.7±5.9
LNCaP	96.8±5.4	90.3±4.6	71.7±2.2	61.5±3.4	45.1±5.0
PC3	96.4±5.0	87.6±4.9	80.1±4.6	75±4.4	73.2±4.1
PC12	91.1±6.5	84.5±6.0	68.5±6.8	57.1±6.3	40.3±4.4
MCF-7	94.8±6.1	90.2±5.9	77.5±5.1	66.4±4.7	47.1±4.2
MOLT-4	88.6±3.6	70.2±4.1	57.5±4.0	46±2.8	42.8±3
U266B1	95.1±4.9	73.1±5	53.8±4.6	47.5±3.3	33.8±4.7
Raji	85.6±4.1	80.1±3.2	68.1±2.8	42.9±3.2	29.4±4.6
CHO	97.7±5.2	91.3±5.5	74.4±4.1	75.4±4.1	70.5±5.2

B, Cells treated for 48 h

Cell line	10 μM	20 μM	40 μM	80 μM	120 μM
CT-26	87.4±5.4	77.7±5.9	70.3±4.1	61.5±3.2	42.1±3
LNCaP	91.5±6.3	84.2±5.1	66.6±5.7	46.7±4.9	38.5±3.8
PC3	89.9±3.6	77.6±3.2	70.7±2.8	52.2±3.3	28.5±3.4
PC12	94.4±5	80.8±3.4	62.5±4.6	44.5±3.2	30.7±3.8
MCF-7	81.3±4.1	70.2±3.1	55.5±3.4	39.6±3.7	25.2±2.1
MOLT-4	70.6±2.8	52.5±2.6	43.1±1.9	33.3±2.5	21.6±1.4
U266B1	68.5±2.3	53.4 ±1.8	37.2±2	33.4±1.6	20.4±2.1
Raji	60.6±3.6	49.5±2.3	30.3±2.4	26.4±2.3	14.6±3.3
CHO	97.4±4.4	81.3±3.4	64.6±2.8	45.8±2.6	21.9±3.5

C, Cells treated for 72 h

Cell line	10 μM	20 μM	40 μM	80 μM	120 μM
CT-26	65.5±1.5	55.8±1.9	35.9±0.83	29.7±1.1	25±2.3
LNCaP	58.4±2.9	51.4±2.6	39±1.9	36.1±2.2	30.7±2
PC3	61.7±2.1	57.3±1.8	46.9±1.4	36.2±0.9	31.5±3.7
PC12	50.4±3.6	47.2±2.6	40.8±1.8	31.9±2.3	22.1±1.1
MCF-7	51.6±3.2	48.5±2.9	35.7±2.5	30.8±3	19.1±1.4
MOLT-4	11.5±0.5	10.2±0.45	10±0.37	5.2±0.48	2.1±0.9
U266B1	15.9±0.8	13.3±0.65	4.8±0.72	6.8±1.1	5.5±0.38
Raji	5.5±0.4	2.7±0.8	1.3±0.25	0.25±0.12	0.18±0.09
CHO	57.8±3.9	52.4±3.2	39.2±2.7	28.5±2.1	20.7±3.7

TUNEL assay. At the end of the experiment (36 days following treatment), all surviving animals were sacrificed, and tumors from all animals were dissected and the poly-D-lysine-coated coverslips for TUNEL assay were positioned. An increase in the percentage of apoptotic cells in the treated as compared to the control groups was observed; however, it did not reach statistical significance (P>0.05; Fig. 5).

Discussion

In spite of many advances in cancer therapy, cancer is still one of the major causes of mortality worldwide. Natural products, particularly flavones found in the human diet, have been found to exert anti-proliferative and apoptosis-promoting effects against cancer cells (60,61). The current study demonstrated

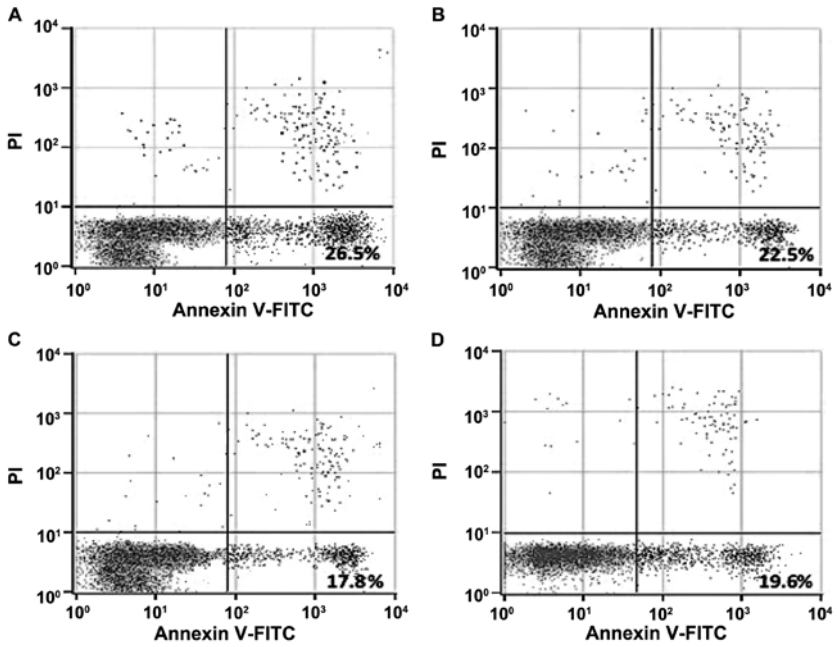

Figure 1. Apoptotic rate determined by Annexin V/PI staining in (A) CT-26, (B) LNCaP, (C) MOLT-4 and (D) Raji cell lines following 48 h of treatment with quercetin at 120 μM. Early apoptotic cells are Annexin V-positive and PI-negative (lower right quadrant). PI, propidium iodide.

Table II. IC_{50} values (in μM) for the studied cell lines following treatment with various concentrations of quercetin (10, 20, 40, 80, 120 μM) for 24, 48 and 72 h.

Cell line	24 h	48 h	72 h
CT-26	118.1±5.55	97.5±4.31	27.2±1.52
LNCaP	110.7±4.30	72.6±5.15	21.7±2.31
PC3	>120	81.9±3.27	36±1.98
PC12	99.3±6.11	65.2±4	11.8±2.27
MCF-7	105.4±5.2	52.5±3.28	13.7±2.61
MOLT-4	64.9±3.5	28.6±2.23	2.91±0.54
U266B1	54.3±4.5	25±1.96	6.13±0.73
Raji	66.5±3.57	19.2±2.83	3.52±0.46
CHO	>120	70.7±3.44	23.4±3.11

IC_{50} values were calculated as previously described by Entezari Heravi *et al* (59).

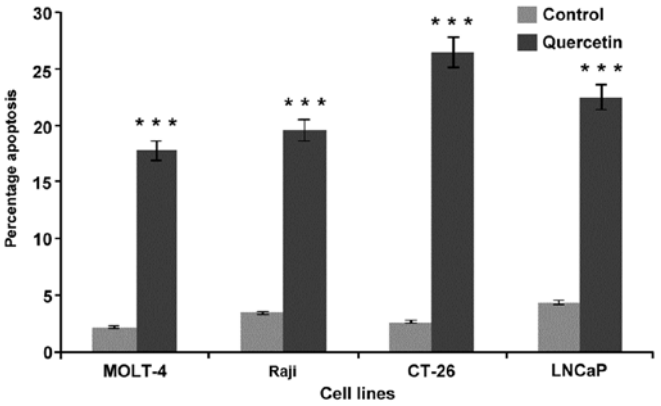

Figure 2. Induction of apoptosis in LNCaP, CT-26, MOLT-4 and Raji cell lines treated with quercetin as assessed by flow cytometer Annexin V/PI. The results revealed that quercetin significantly induced apoptosis in comparison to the control group (***P<0.001). PI, propidium iodide.

that quercetin induces the apoptosis of various cancer cell lines. Furthermore, a significant increase in the survival rate and a significant reduction in tumor volume was observed in tumor-bearing animals treated with quercetin.

Previous studies have shown that grape stem extracts have an ability to inhibit the growth of colon (HT29), breast (MCF-7), renal (Caki-1) and thyroid (K1) cancer cell lines (62,63). These extracts are rich in flavonols, particularly quercetin and rutin (63). Importantly, quercetin was found to be in both aglycon and glycoside forms (64). The inhibitory effect of quercetin on cancer cell growth is attributed to the inhibition of survival signaling proteins, such as protein kinase C (PKC-α) and the activation of death signals, such as PKC-δ (63). Moreover, grape stem extracts seem to present an anti-angiogenic potential evident by VEGF downregulation (65).

Furthermore, quercetin induces pro-apoptotic effects via different mechanisms involving antioxidant effects and the suppression of p53 gene and BCL-2 protein (66). The suppression of BCL-2 gene transcription diminishes the inhibitory effects on BAD protein in the mitochondria, which is considered as the initiator of apoptosis for the intrinsic pathway (67).

The role of quercetin in apoptosis mediated by p53 has been studied in many cancer cell lines. When p53 is inhibited, cells become more susceptible toward cytotoxicity induced by quercetin (68). Apart from cell cycle regulation and the induction of apoptosis, p53 acts as a modulator of intracellular levels of ROS. In this regard, p53 exerts antioxidant effects in cells with no or low stress through the regulation of genes involved in such activity, which comprises microsomal GSH

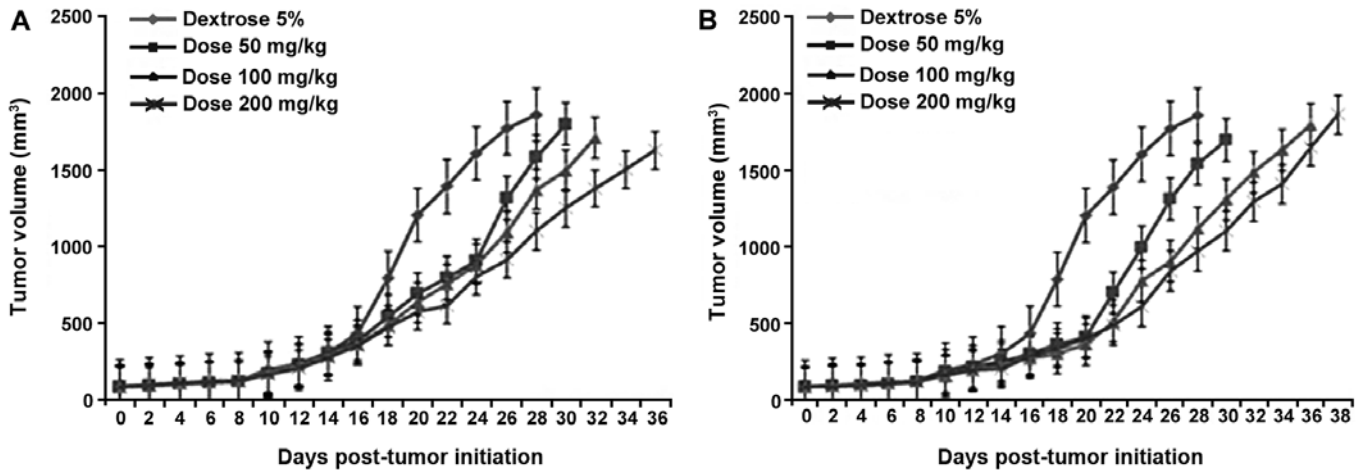

Figure 3. (A) *In vivo* tumor growth inhibition in BALB/c mice treated with various doses of quercetin (50, 100 and 200 mg/kg;intraperitoneally) inducted CT26 and (B) MCF-7 cell line. There were significant differences after 18 days of treatment between the quercetin-treated groups and the control group (dextrose 5%). P<0.001.

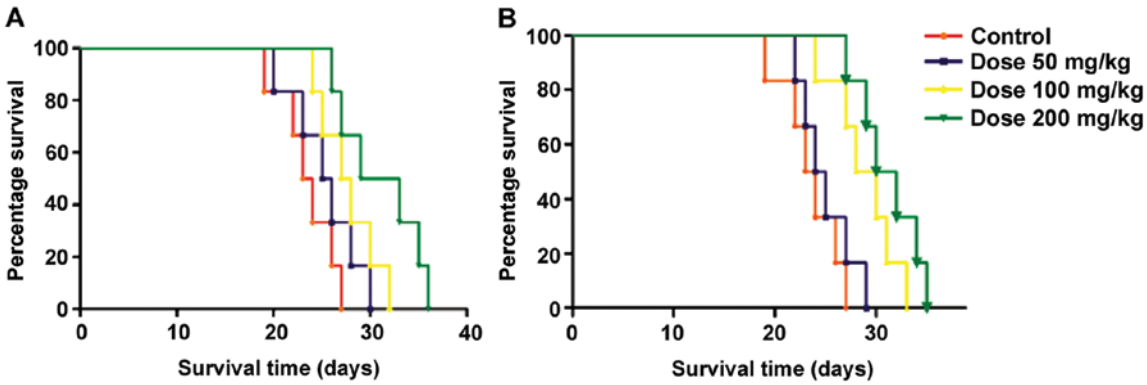

Figure 4. *In vivo* therapeutic efficacy of quercetin in BALB/c mice bearing (A) CT26 and (B) MCF-7 tumors following the intraperitoneal injection of quercetin at multiple doses (50, 100 and 200 mg/kg).

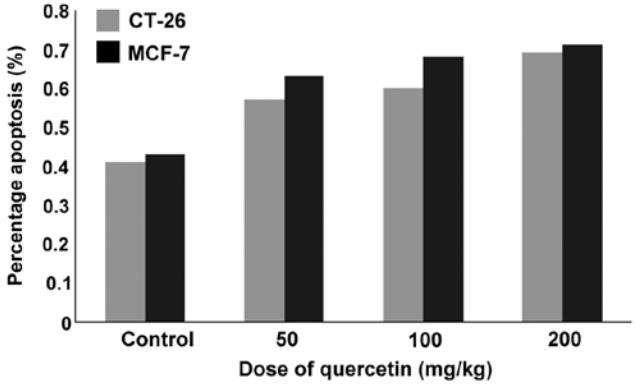

Figure 5. Percentage apoptosis in tumor slices from mice bearing CT-26 and MCF-7 tumors treatment with various concentration of quercetin (50, 100 and 200 mg/kg; intraperitoneally) as assessed by TUNEL assay.

transferase homolog PIG12 (69), aldehyde dehydrogenase 4 family member A1 (ALDH4A1) (70), Gpx1, manganese superoxide dismutase (SOD2) (71) and catalase (72). Some studies, however, have suggested that the effect of quercetin may be independent of p53. Although apoptotic cell death caused by DNA damage is often mediated by p53, there are other proteins, such as p63 and p73, which may be involved in this mechanism (73). Chien *et al* demonstrated that quercetin-induced apoptotic cell death was accompanied by a decrease in p53 expression in breast cancer cells (74). Additionally, quercetin inhibited the metabolic activity and induced cell death by apoptosis, followed by an increase in BAX expression with a concomitant decrease in the expression of anti-apoptotic proteins.

Quercetin has also been found to modulate the PI3K/Akt/mTOR pathway (75). The flavonoid has structural homology to the PI3K inhibitor, LY294002 (LY) and as expected, the phytochemical was found to inhibit the PI3K-Akt pathway in a similar manner to the inhibition elicited by LY in the breast cancer cell lines, HCC1937 and T47D (76). Breast cancer invasion was found to be suppressed by quercetin through the inactivation of the PKC/ERK/AP-1-dependent MMP-9 activation (76).

Quercetin exerts anticancer effects through the cell death domain mechanism at the cell surface (67). Quercetin activated the cell death domain which leads to FAS and FADD activation, and the induction of cell death in a cancer cell line via activation of caspase 8 (67). The above-mentioned findings are in accordance with the findings of the present study, which

demonstrated the apoptotic-inducing properties of quercetin as assessed by the Annexin V/PI method.

The expression of heat shock proteins (HSPs) in almost all forms of cancer is elevated (58). Badziul *et al* showed that quercetin decreased the transcription and translation of HSP27 and 72 in the T98G cell line (58). HSPs are involved in cell proliferation and the inhibition of their production leads to cell apoptosis (77). This is well in accordance with our study, as we demonstrated that apoptosis was induced in quercetin-treated cancer cell lines. HSP27 has been reported to promote the development of leukemia by protecting tumor cells from apoptosis through various mechanisms. Another study investigated the effects of small hairpin (sh)RNA-mediated HSP27 knockdown on the anticancer effects of quercetin in U937 human leukemia cells. The results indicated that shHSP27 and quercetin synergistically inhibited U937 cell proliferation and induced apoptosis by reducing the Bcl2/Bax ratio. Moreover, this combined treatment significantly suppressed the infiltration of tumor cells and the expression of the angiogenesis-associated proteins, hypoxia-inducible factor 1α (HIF1α) and vascular endothelial growth factor (VEGF). In comparison with shHSP27 or quercetin separately, shHSP27 and quercetin together, notably decreased the expression of cyclin D1, and thus the cell cycle was arrested at the G1 phase (78). Furthermore, the Notch/AKT/mTOR signaling pathway contributes to tumor aggressiveness; quercetin plus shHSP27 has been shown to significantly decrease Notch 1 expression and the phosphorylation levels of the downstream signaling proteins, mTOR and AKT (79).

The anticancer effects of quercetin have been confirmed in many studies (36,42,44). Specifically, *in vitro* and *in vivo* studies have suggested that quercetin possesses anticancer activity against different tumors; e.g. colon, lung, breast and prostate cancer (78,80-84). Quercetin can exert its anticancer effects through different mechanisms, including the inhibition of DNA topoisomerase I/II, the release of cytochrome *c*, the activation of caspase 3, and HSP27 and 72 elevation (37,58,85).

An *in vivo* examination of the effects of quercetin in mice bearing CT-26 tumors was performed for the first time in this study, at least to the best of our knowledge. The results revealed that quercetin significantly reduced the tumor volume and increased animal survival. Previous studies have provided evidence for the anticancer effects of quercetin on breast and prostate cancers *in vivo* (86,87), an observation which was verified in the current study using the MCF-7 breast cancer *in vivo* model.

Importantly, quercetin can exert tumor suppressive effects by interfering with the cell cycle. The molecular targets of this flavonoid include p27, topoisomerase II, p21 and cyclin B (88,89). Depending on the tumor origin, quercetin is able to block the cell cycle at G2/M or at the G1/S transition (90,91). In breast cancer cell lines, a low dose of quercetin has been shown to induce mild DNA damage and Chk2 activation, which is the main regulator of p21 expression (92). In addition, quercetin has been shown to downregulate cyclin B1 and CDK1, essential components of G2/M cell cycle progression (92). Moreover, quercetin can inhibit the recruitment of NF-Y, a key transcription factor, which binds to the cyclin B1 gene promoter and leads to transcriptional cessation. In the human hepatoma cell line (HepG2), quercetin upregulated p21, p27 and p53, and consequently the cells were arrested at the G1 phase (93).

Furthermore, quercetin has been shown to inhibit NF-κB-evoked pathways of cell survival and reduce pro-inflammatory cytokine expression that finally leads to cancer formation (94). Notably, quercetin inhibits the production of tumor necrosis factor (TNF)-α, a major pro-inflammatory molecule involved in chronic inflammatory diseases, which may develop into tumors. The quercetin-induced suppression of TNF-α results in the stimulation of anti-inflammatory cytokines through the inhibition of NF-κB activation (95). TNF-α is a major regulator of the cellular release of other chemokines, cytokines and other inflammatory mediators, and thus can be considered as a potential target for the treatment of inflammatory diseases and inflammation-driven cancer. In the current study, quercetin was able to suppress tumor growth and improve animal survival (independent of its action on the induction of apoptosis).

Quercetin seems to play an inhibitory role in angiogenesis in human prostate tumor growth. A recent study using an animal model, indicated that low doses of quercetin inhibited the following angiogenic stages: proliferation and migration, as well as the invasion and tube formation of endothelial cells. Protein expression analysis of prostate cancer cells treated with quercetin revealed the inhibition of the VEGF-induced phosphorylation of VEGFR-2 and its downstream targets, such as mTOR, Akt, and ribosomal S6 kinase (86). Thus, quercetin can decrease tumor volume and increase animal survival rate following systemic administration, a finding which is consistent with the results of the present study.

The oral administration of quercetin is recommended for cancer prevention. It has been shown that a diet supplemented with 2% quercetin significantly reduced the onset of colorectal cancer (96). In addition, *in vitro* studies have proved its potency in inhibiting the proliferation of colon cancer cells of different lineages (97,98). However, in a phase-1 clinical study performed at the University of Birmingham for the evaluation of the non-toxic and anticancer efficacy of quercetin in terminally ill cancer patients, no patient achieved conventional radiological response according to the WHO criteria, despite favorable indications of its anticancer activity (99).

In conclusion, our results demonstrate that quercetin inhibits the growth of a panel of 9 cancer cell lines with various IC_{50} values. Cell growth inhibition was attributed to the induction of apoptosis, evident in the CT-26, PC-12, LNCaP and PC-3 cancer cell lines. Furthermore, our results demonstrated that quercetin reduced CT-26 and MCF-7 tumor volume in a mouse model and increased animal survival; however, we did not verify increased *in situ* apoptosis in the induced tumors. The current study results strongly suggest that quercetin has potential for therapeutic application in neoplasia; however, further studies are required to confirm these findings.

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