

# Apoptotic Effects of Quercitrin on DLD-1 Colon Cancer Cell Line

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Received: 28 April 2014 / Accepted: 24 July 2014 / Published online: 6 August 2014  
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**Abstract** Quercetin, which is the most abundant bioflavonoid compound, is mainly present in the glycoside form of quercitrin. Although different studies indicated that quercitrin is a potent antioxidant, the action of this compound is not well understood. In this study, we investigated whether quercitrin has apoptotic and antiproliferative effects in DLD-1 colon cancer cell lines. Time and dose dependent antiproliferative and apoptotic effects of quercitrin were subsequently determined by WST-1 cell proliferation assay, lactate dehydrogenase (LDH) cytotoxicity assay, detection of nucleosome enrichment factor, changes in caspase-3 activity, loss of mitochondrial membrane potential (MMP) and also the localization of phosphatidylserine (PS) in the plasma membrane. There were significant increases in caspase-3 activity, loss of MMP, and increases in the apoptotic cell population in response to quercitrin in DLD-1 colon cancer cells in a time- and dose-dependent manner. These results revealed that quercitrin has antiproliferative and apoptotic effects on colon cancer cells. Quercitrin activity supported with *in vivo* analyses could be a biomarker candidate for early colorectal carcinoma.

**Keywords** Colon Cancer · Quercitrin · Antiproliferative · Apoptosis

## Introduction

Colorectal cancer (CRC) is the third most common cancer and the second highest cause of cancer-related death worldwide with rapidly increasing rates due to many risk factors including smoking, physical inactivity, obesity, red processed meat and excessive alcohol consumption [1]. Progress in diagnosis and treatment has had a positive effect on improving overall survival, with more patients being diagnosed in the early stage of the disease, but the outcomes of patients diagnosed with advanced stage disease remains quite poor [2]. Understanding the molecular genesis of CRC is essential for identification of novel molecular targets that might be useful to define the prognosis of CRC patients [3]. Different methods have been introduced to substantially reduce the incidence and mortality of colon cancer cell lines. The search for new compounds having anti-cancer effects in foods and plant medicines gives rise to realistic and promising approach for treatments [4]. A variety of compounds have undergone clinical trials against colon cancer based on this strategy [5, 6]. Flavonoids are naturally occurring molecules that are abundant in fruits, vegetables, nuts, seeds, and beverages such as tea and wine. Studies have shown various biological activities of flavonoids as inhibiting related cancer enzymes, antioxidant, and immune response activities [7–11]. These properties could explain the beneficial effects of these natural molecules that have already been used in hypertension, inflammation, and cancer treatments [12]. Quercetin in the glycoside form is the most abundant bioflavonoid compound [13]. Quercitrin is a flavonoid with antiinflammatory activity in experimental colitis, associated with an antioxidative action [14]. Recent studies demonstrated that quercitrin exhibits

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scavenger and anti-oxidant properties that increase the potential for quercitrin being an anti-carcinogenic [15]. Although different studies indicated that quercitrin is a potent antioxidant, the action of this compound is not well understood [16–18]. Hence, we proposed to investigate whether quercitrin has apoptotic and antiproliferative effects in DLD-1 colon carcinoma cell lines.

## Material & Methods

### Cell Culture

The Dukes' type C colorectal adenocarcinoma carcinoma cell line DLD-1 was kindly provided by Dr. Yusuf Baran (Department of Molecular Biology and Genetics, IYTE). DLD-1 cell line was cultured in DMEM medium containing 1 % penicillin/streptomycin and 10 % fetal bovine serum at 37 °C in 5 % CO<sub>2</sub>.

### Measurement of Cell Growth and Cytotoxicity

To detect the effect of quercitrin on cell viability after treatment, a WST-1 cell proliferation assay was performed. The WST-1 conversion assay (Roche, Germany) is based on the mitochondrial function of intact cells that enables them to metabolise the stable tetrazolium salt WST-1 (4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene disulfonate) to a soluble violet formazan product. Quantities of  $1 \times 10^4$  cells/well were seeded into 96-well plates containing 100  $\mu$ l of the growth medium in the absence or presence of increasing concentrations of quercitrin and then incubated at 37 °C in 5 % CO<sub>2</sub> for 24, 48 and 72 h. After the incubation period, cells were treated with 10  $\mu$ l of WST-1 for 4 h. Dye accumulation was measured at 450 nm using a Multiscan ELISA reader (Thermo Fisher Scientific, Germany). Viability was calculated by subtraction of the mean values without WST-1 from those with WST-1 substrate and was expressed as a percentage of control. Data was confirmed by three other independent experiments.

Cytotoxic effects of quercitrin in the dose and time dependent manner were colorimetrically determined with a "CytoTox 96R Non-Radioactive Cytotoxicity Assay" kit from Promega (Madison, WI). Cell treatment to prepare for cytotoxicity test was done as described for the WST-1 assay. Culture medium (10  $\mu$ l) was then transferred to a 96-well microtiter plate. The levels of lactate dehydrogenase (LDH) were determined by adding 50  $\mu$ l fresh substrate mix, incubating in a dark at room temperature for 30 min, then adding 50  $\mu$ l stop solution, and measuring optical density (OD) at 490 nm with a microplate reader (Bio-Rad, Hercules, CA). The natural color of chemicals at

490 nm was corrected by subtracting the OD values of the corresponding chemical  $\times$  concentration medium that were treated and measured in triplicates in the same manner as with the cells. Data was confirmed by three other independent experiments.

### Detection of Apoptotic Nucleosomes and Necrotic DNA Release

The Cell Death Detection Elisa plus (CDDE) kit is a photometric enzyme-immunoassay to determine the quality and quantity of DNA in terms of necrosis and cytoplasmic histone-associated DNA fragments (mono- and oligonucleosomes) related to apoptosis in the supernatant of treated cells (Roche, Germany). Application according to manufacturer's instruction provides concurrent detection of apoptosis and necrosis in the same well. Colour development of samples was determined by enrichment factor of the amount of DNA fragments in cytoplasm or cell supernatant indicating apoptosis or necrosis, respectively, and expressed relative to untreated cells. In order to investigate possible interference with the assay, particles were added to the cells as two fold higher than the final concentration used in above experiment (160  $\mu$ g/cm<sup>2</sup>). Subsequently, the samples were centrifuged (10 min, 200 g) conformance with the CDDE kit protocol. The supernatant was then mixed 1:1 with lysate of 50  $\mu$ m quercitrin treated cells for 48 and 72 h using the lysis buffer included in the kit, and analysed according to manufacturer's instructions.

### Analysis of Phosphatidylserine Exposure and Cell Permeability

During apoptotic cell death, phosphatidylserine (PS), which is a phospholipid component of the inner-leaflet of cell membrane, becomes available at the cell surface. This early marker of apoptotic cell death can be detected by staining with the green fluorescent dye Annexin V-FITC (BD Pharmingen, Germany), a fluorochrome-conjugated Ca<sup>2+</sup>-dependent PS-binding protein. In combination with the 7-AAD-DNA-staining, dye exclusion of vital cells permits a discrimination between apoptotic and necrotic cells. Cells treated in 24-well dishes were centrifuged (200 g, 5 min, 4 °C), washed with HBSS<sup>-/-</sup> (GIBCO, Germany) and stained with 150  $\mu$ l of buffer (10 mM HEPES/NaOH pH 7.4, 150 mM NaCl, 5 mM KCl, 2 mM CaCl) containing 5  $\mu$ l Annexin V-FITC (1 mg/ml) and 1.5  $\mu$ M 7-AAD at 37 °C. After 10 min, an additional 500  $\mu$ l of ice-cold buffer was added by the time the cells were in the ice. After centrifugation (200 g, 5 min, 4 °C) cells were suspended in 250  $\mu$ l buffer and analysed immediately by flow cytometry using the green-collecting fluorescent channels FL-1 for Annexin V-FITC and FL-3 for 7-AAD, as described above. Quadrant separation in the fluorescent

channels FL-1 (Annexin V-FITC) versus FL-3 (7-AAD) represents events of necrotic and apoptotic cells. 7-AAD positive (Q1+Q2); apoptotic cells (Q3, Annexin V-FITC positive and 7-AAD negative) and viable cells (Q4, fluorescent negative) were expressed as percentage of total cells.

#### Analysis of Caspase-3 Enzyme Activity

Changes in caspase-3 enzyme activity of the cells, which are important signs of apoptosis, were examined using the caspase-3 colorimetric assay kit (BioVision Research Products, USA). This assay is based on spectrophotometric detection of the chromophore p-nitroanilide (pNA) after cleavage from the labeled substrate DEVD-pNA, which can be recognized by caspase-3. In short, the cells ( $5 \times 10^5$  cells/2 mL/well) that were induced to undergo apoptosis were collected by centrifugation at 1,000 rpm for 10 min. The cells were lysed by adding 50  $\mu$ l of chilled cell lysis buffer and incubated on ice for 10 min before centrifugation at 10,000 g for 1 min. Supernatants were transferred to new Eppendorf tubes, and 150  $\mu$ l of cell lysis buffer was added to dilute the samples. The reaction mixture was prepared in 96-well plates by adding 50  $\mu$ l of reaction buffer (containing 10 mM DTT), 50  $\mu$ l of sample, and 5  $\mu$ l of DEVD-pNA substrate and incubated for 2 h at 37 °C in CO<sub>2</sub> incubator. At the end of this period, the plate was read under 405 nm wavelengths using an Elisa reader (Thermo Electron Corporation Multiskan Spectrum, Finland). The absorbance values are normalized to protein concentrations determined using a Bradford assay as described previously.

#### Determination of Changes in Mitochondrial Membrane Potential

We also examined the loss of MMP, another important sign of apoptosis, in response to quercitrin treatment for 48 h in DLD-1 and MRC5 cells using the JC-1 Mitochondrial Membrane Potential Detection Kit (Cayman Chemicals, USA). This kit uses JC-1, a unique cationic dye, to signal the loss of the MMP. JC-1 accumulates in the mitochondria which stain red in nonapoptotic cells, while in apoptotic cells the MMP collapses, and thus the JC-1 remains in the cytoplasm as a monomer that stains green under fluorescent light. The cells ( $5 \times 10^5$  cells/2 mL) were induced to undergo apoptosis and collected by centrifugation at 1,000 rpm for 10 min. Supernatants were removed, pellets were homogenized by 200  $\mu$ l of medium, and 20  $\mu$ l of JC-1 dye was added onto the cells; then, the cells were incubated at 37 °C in 5 % CO<sub>2</sub> for 30 min. Then, they were centrifuged at 400 g for 5 min, supernatants were removed, and 200  $\mu$ l of assay buffer was added onto the pellets and vortexed. Then, this step was repeated once more. Afterwards, all pellets were resuspended with 320  $\mu$ l assay buffer and 100  $\mu$ l from each of

them was added into the 96-well plate as triplicates. In healthy cells, the aggregate in red form has absorption/emission maxima of 560/595 nm, whereas in apoptotic cells the monomeric green form has absorption/emission maxima of 485/535 nm. The plate was read at these wavelengths using a fluorescence Elisa reader (Thermo Varioskan Spectrum, Finland). The green/red (485/560) values were calculated to determine the changes in MMP.

#### Statistical Analysis

Results are expressed as the mean standard error of the mean (SEM). The data were analyzed using one-way ANOVA.

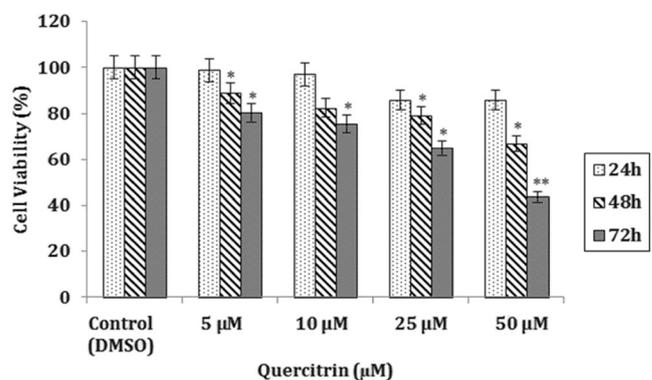
## Results

### Quercitrin Inhibited Proliferation of DLD-1 Colorectal Carcinoma Cell Line in a Time- and Dose-Dependent Manner

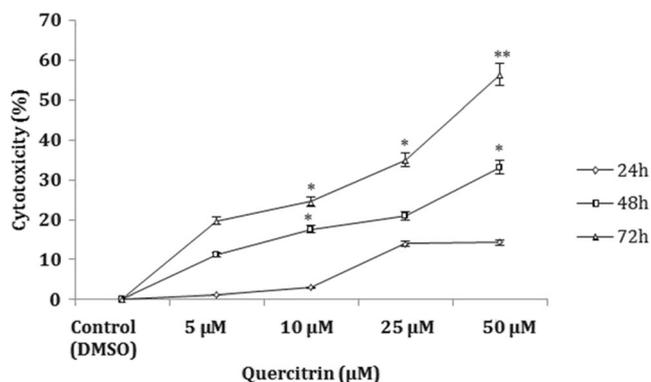
To determine the antiproliferative effects of quercitrin on DLD-1 colorectal carcinoma, the cells were incubated with increasing concentrations of quercitrin for 24, 48, 72 h using a WST-1 cell proliferation assay. The results of these assays showed that there were time- and dose- dependent decreases in cell proliferation when compared with controls only in the colorectal carcinoma cell line (Fig. 1).

### Quercitrin has Cytotoxic Effects on DLD-1 Colorectal Carcinoma Cell Line in a Time- and Dose-Dependent Manner

To determine the cytotoxic effects of quercitrin on DLD-1 colorectal carcinoma, the cells were incubated with increasing concentrations of quercitrin for 24, 48, 72 h using LDH assay. The results of these assays showed that there were time- and dose- dependent increases in cytotoxicity. (Fig. 2).



**Fig 1** Effects of quercitrin on cell proliferation of DLD-1 cells. The WST-1 assays were performed using triplicate samples in three independent experiments. Statistical significance was determined using two-way analysis of variance,  $p < 0.05$  (\*) and  $p < 0.01$  (\*\*) were considered significant



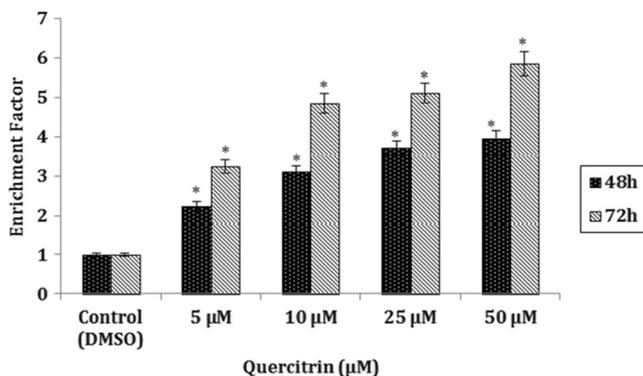
**Fig 2** Effects of quercitrin on cell cytotoxicity of DLD-cells. The LDH cytotoxicity assays were performed using triplicate samples in three independent experiments. Statistical significance was determined using two-way analysis of variance, and  $p < 0.05$  (\*) and  $p < 0.01$  (\*\*) were considered significant

### Quercitrin Increases Nucleosomal Enrichment Factor in Time and Dose Dependent Manner

To determine the apoptotic effects of quercitrin on DLD-1 cells, these cells were incubated with increasing concentrations of quercitrin for 48 and 72 h and changes in nucleosomal enrichment factor (EF) were analyzed. There were subsequently 3.95 and 5.85 fold increases in EF in response to 48 and 72 h incubation with 50 µM quercitrin on DLD-1 cells, as compared with untreated cells (Fig. 3).

### Quercitrin Increases Caspase-3 Enzyme Activity in Time and Dose Dependent Manner

To determine the apoptotic effects of quercitrin on DLD-1 cells, these cells were incubated with increasing concentrations of quercitrin for 48 and 72 h. Changes in caspase-3 enzyme activities were analyzed. There was 1.5 fold increases in caspase3 activity in response to 48 h incubation with 50 µM



**Fig 3** Effects of quercitrin on apoptosis related nucleosomal enrichment factor of DLD-1 cells. The Cell Death detection plus ELISA were performed using triplicate samples in at least two independent experiments. Statistical significance was determined using two-way analysis of variance, and  $p < 0.05$  was considered significant

quercitrin on DLD-1 cells, as compared with untreated cells (Fig. 4). Quercitrin induced apoptosis in a dose-dependent manner could be related with caspase-3 activity.

### Quercitrin Induces the Loss of Mitochondrial Membrane Potential in a Time- and Dose- Dependent Manner

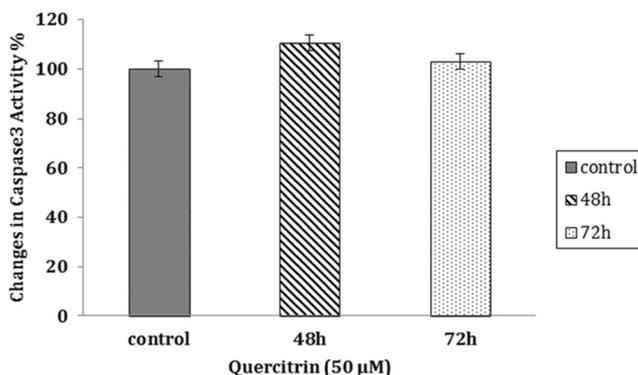
To assess the loss of MMP, DLD-1 cells, a JC-1 MMP assay was performed after the cells were exposed to 50 µM quercitrin for 48,72 h. The results of this assay revealed the 1.8 and 1.36 fold increases in loss of MMP in response to 50 µM quercitrin for 48 and 72 h DLD-1 cells respectively, as compared with untreated cells (Fig. 5).

### Quercitrin Causes the Modulation of the Cell Membrane Resulting in the Translocation of PS from the Inner to Outer Leaflet in a Time and Dose Dependent Manner

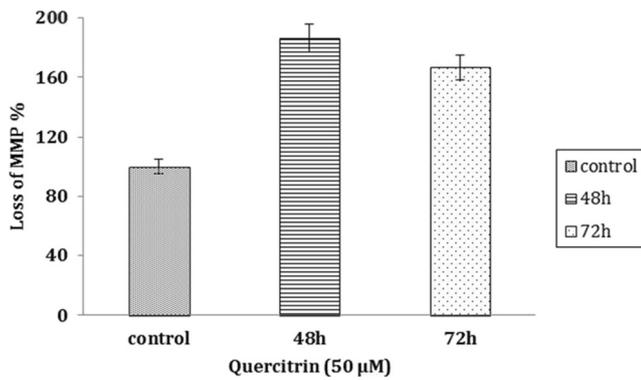
In order to confirm the results of caspase-3 activity and loss of MMP, FITC AnnexinV/PI double staining was performed in DLD-1 cells and The cells were exposed to 50 µM quercitrin for 48 h was done. The results demonstrated that 50 µM quercitrin increased the apoptotic cell death as compared with the untreated control group (Fig. 6–7).

## Discussion

Quercetin is the most ubiquitous flavonoid in nature, including foods, which is usually present in a glycosylated form such as quercitrin. It possesses various potentials as an antioxidant, anti-inflammatory; however, the underlying mechanisms leading to its anticancer effects is still unclear. Comalada et al. analyzed the potency and efficacy of both quercetin and quercitrin as anti-inflammatory compounds. They examined the in vitro effects of quercitrin in the



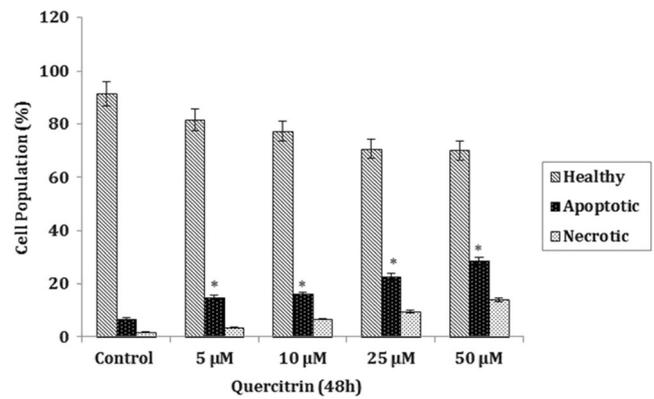
**Fig 4** Effects of quercitrin on caspase3 activity in DLD-1 cells. Changes in caspase-3 enzyme activity in response to increasing concentrations of quercitrin in DLD-1 cells. The results are the means of two independent experiments.  $p < 0.05$  was considered significant



**Fig 5** Effects of quercitrin on membrane potential in DLD-1 cells. Loss of mitochondrial membrane potential in response to increasing concentrations of quercitrin in DLD-1 cells. The results are the means of two independent experiments.  $p < 0.05$  was considered significant

initiation and progression of the inflammation process in the oxidative stage of colon carcinoma. Upregulated nitric oxide synthase was inhibited in these rat colitis, in addition NF- $\kappa$ B inactivation was detected [19].

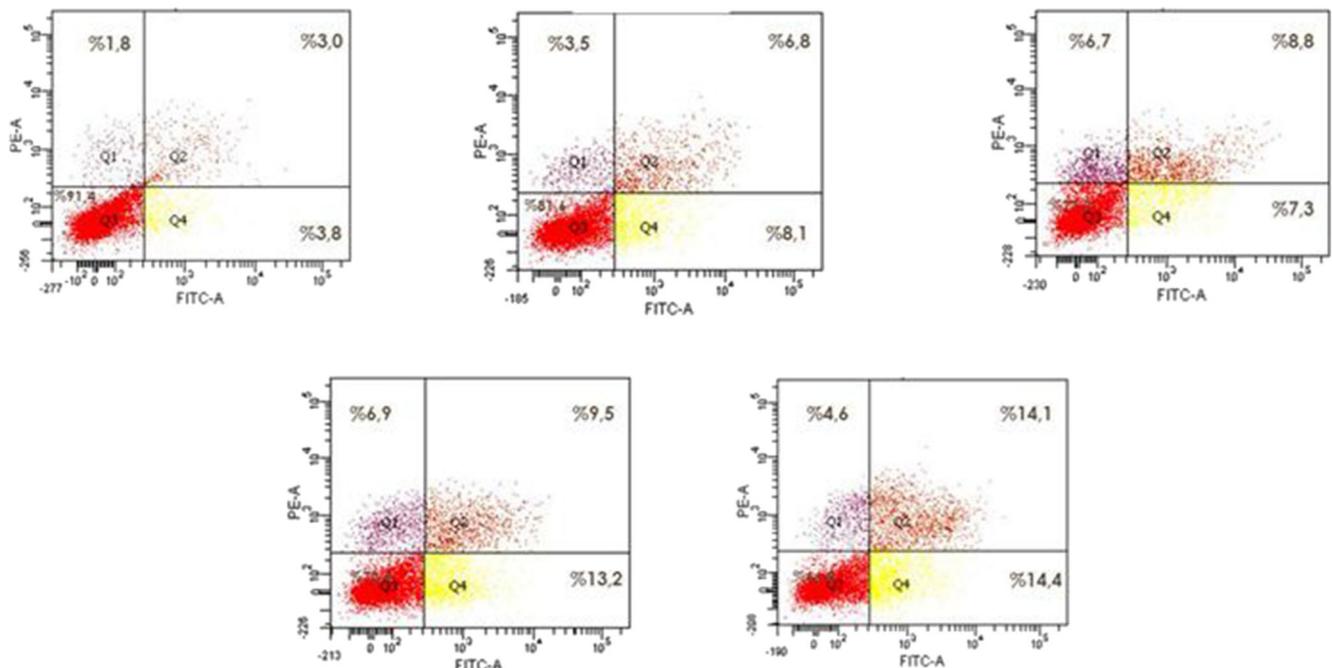
The research on macrophage activity of quercetin and quercitrin indicated that quercetin, instead of quercitrin, is able to repress the proliferation and activation of macrophages by inhibiting cytokine and nitric oxide formation, thus down regulating the NF $\kappa$ B pathway [20]. This study in rat colitis confirmed the protective and therapeutic activity of quercitrin [21]. Ding et al. examined the effects of quercitrin on neoplastic JB6 cells, the results demonstrated that the TPA-



**Fig 7** Effects of quercitrin (0 to 50 microM) on apoptosis in DLD-1 cells. FITC AnnexinV/PI double staining of DLD-1 cells treated with increasing concentrations of quercitrin (a). Flow cytometric analysis of apoptosis in DLD-1 cells (b). Early apoptotic cells labelled with Annexin-V but not PI (shown in lower right quadrant) and apoptotic cells labelled with Annexin-V and PI were found in the upper right quadrant in flow cytometric graphics. The results are the means of two independent experiments.  $p < 0.05$  was considered significant

induced neoplastic transformation is blocked in tumor cells. They also showed that quercitrin stimulated the activation of NF-E2-related factor (Nrf2), which is essential in the regulation of cellular protective genes, and blocked damage in DNA due to UVB [13].

Despite much inflammatory and protective cell researches, there are limited studies about the anticancer effects of quercitrin in the literature. Epidemiologic studies have



**Fig 6** Effects of quercitrin (0 to 50 microM) on apoptosis in DLD-1 cells. FITC AnnexinV/PI double staining of DLD-1 cells treated with increasing concentrations of quercitrin (a). Flow cytometric analysis of apoptosis in DLD-1 cells (b). Early apoptotic cells labelled with Annexin-V but not

PI (shown in lower right quadrant) and apoptotic cells labelled with Annexin-V and PI were found in the upper right quadrant in flow cytometric graphics. The results are the means of two independent experiments.  $p < 0.05$  was considered significant

indicated that dietary flavonoids could have a role in oral cancer. Brownning et al. examined whether quercitrin directly affected cell proliferation in a human oral squamous carcinoma cell line. They found that quercitrin had no response to antiproliferative activity as a result of minimal cellular uptake and no hydrolysis of glycosides [22]. Xu and et al. studied the molecular mechanisms of apoptosis on a prostate cancer cell line PC3 induced by quercitrin and found that quercitrin possessed little effect on inducing apoptosis [23].

In this study, we determined that quercitrin has antiproliferative, cytotoxic and apoptotic effects as would be desirable for a strategy for in cancer chemotherapy on colon carcinoma cell lines in a dose and time dependent manner. Also, we found that quercitrin exerts an apoptotic effect through the loss of mitochondrial membrane potential. Nonfunctional mitochondria played a role in the induction of apoptosis and could be essential in the apoptotic pathway [24]. We hypothesized that quercitrin affects colon cancer cell lines by immune modulation.

In conclusion, taken together, all these data showed the antiproliferative and apoptotic effects of quercitrin on colon cancer cells. Based on the above results, quercitrin appears to activate specific intracellular death-related pathways in DLD-1 cells, leading to a loss of mitochondrial cell potential and inducing apoptosis. Our findings suggest the possible use of quercitrin against human colon cancer.

**Acknowledgments** This work was supported by the Istanbul University Scientific Research Projects; 31162. We would like to thank to Mr. David Chapman for English editing.

**Conflict of interest** The authors declare that they have no financial disclosures or conflicts of interest.

## References

- Jemal A, Bray F, Center MM et al (2011) Global cancer statistics. *CA Cancer J Clin* 61:69–90
- Akao Y, Nakagawa Y, Naoe T (2006) MicroRNAs 143 and 145 are possible common onco-microRNAs in human cancers. *Oncol Rep* 16:845–850
- Valeri N, Gasparini P, Fabbri M et al (2010) Modulation of mismatch repair and genomic stability by miR-155. *Proc Natl Acad Sci U S A* 107:6982–6987
- Wattenberg LW (1985) Chemoprevention of cancer. *Cancer Res* 45:1–8
- Boone CW, Kelloff GJ, Malone WE (1990) Identification of candidate cancer chemopreventive agents and their evaluation in animal models and human clinical trials: a review. *Cancer Res* 50:2–9
- Reddy BS (2000) The Fourth DeWitt S. Goodman lecture. Novel approaches to the prevention of colon cancer by nutritional manipulation and chemoprevention. *Cancer Epidemiol. Biomark Prev* 9:239–247
- Cody V (1988) Crystal and molecular structures of flavonoids. *Prog Clin Biol Res* 280:29–44
- Pietta PG (2000) Flavonoids as antioxidants. *J Nat Prod* 63:1035–1042
- Tripoli E, Guardia ML, Giammanco S et al (2007) Citrus flavonoids: molecular structure, biological activity and nutritional properties: a review. *Food Chem* 104:466–479
- Pilsakova L, Riečanský I, Jagla F (2010) The physiological actions of isoflavone phytoestrogens. *Physiol Res* 59:651–664
- Domitrović R (2011) The molecular basis for the pharmacological activity of anthocyanins. *Curr Med Chem* 18:4454–4469
- Tanaka T, Takahashi R (2013) Flavonoids and asthma. *Nutrition* 5(6):2128–43
- Ding M, Zhao J, Bowman L et al (2010) Inhibition of AP-1 and MAPK signaling and activation of Nrf2/ARE pathway by quercitrin. *Int J Oncol* 36(1):59–67
- Sánchez de Medina F, Vera B, Gálvez J et al (2002) Effect of quercitrin on the early stages of hapten induced colonic inflammation in the rat. *Life Sci* 70:3097–3108
- Gee JM, DuPont MS, Rhodes MJ et al (1998) Quercetin glucosides interact with the intestinal glucose transport pathway. *Free Radic Biol Med* 25:19–25
- Wagner C, Fachinetto R, Dalla Corte CL et al (2006) Quercitrin, a glycoside form of quercetin, prevents lipid peroxidation in vitro. *Brain Res* 1107:192–198
- Cruz EA, Da-Silva SA, Muzitano MF et al (2008) Immunomodulatory pretreatment with *Kalanchoe pinnata* extract and its quercitrin flavonoid effectively protects mice against fatal anaphylactic shock. *Int Immunopharmacol* 8(12):1616–21
- Yang HM, Ham YM, Yoon WJ, Roh SW, Jeon YJ et al (2012) Quercitrin protects against ultraviolet B-induced cell death in vitro and in an in vivo zebrafish model. *J Photochem. Photobiol. B Sep* 3;114:126–31
- Camuesco D, Comalada M, Rodríguez-Cabezas ME et al (2004) The intestinal anti-inflammatory effect of quercitrin is associated with an inhibition in iNOS expression. *Br J Pharmacol* 143:908–918
- Comalada M, Camuesco D, Sierra S et al (2005) In vivo quercitrin anti-inflammatory effect involves release of quercetin, which inhibits inflammation through down-regulation of the NF-kappaB pathway. *Eur J Immunol* 35(2):584–92
- Sánchez de Medina F, Vera B, Gálvez J, Zarzuelo A (2002) Effect of quercitrin on the early stages of hapten induced colonic inflammation in the rat. *Life Sci* 70(26):3097–108
- Browning AM, Walle UK, Walle T (2005) Flavonoid glycosides inhibit oral cancer cell proliferation—role of cellular uptake and hydrolysis to the aglycones. *J Pharm Pharmacol* 57(8):1037–42
- Xu R, Zhang Y, Ye X, Xue S, Shi J et al (2013) Inhibition effects and induction of apoptosis of flavonoids on the prostate cancer cell line PC-3 in vitro. *Food Chem* 138(1):48–53
- Ly JD, Grubb DR, Lawen A (2003) The mitochondrial membrane potential ( $\Delta\psi(m)$ ) in apoptosis: an update. *Apoptosis* 8(2):115–28