OSMANGAZİ TIP DERGİSİ 37(2), 14-20 ORJİNAL MAKALE
Mayıs 2015

How does Quercetin and Tamoxifen Affect the Apoptosis of Colon Cancer Cells?

[1]Didem Turgut Cosan, [1]Ahu Soyocak, [1]Ayse Basaran, [1]İrfan Değirmenci, [1]Hasan Veysi Güneş, [2]Cengiz Bal

[1]Eskisehir Osmangazi University, Faculty of Medicine, Department of Medical Biology, Eskisehir
[2]Eskisehir Osmangazi University, Faculty of Medicine, Department of Biostatistics, Eskişehir
*email: dcosan@ogu.edu.tr

ABSTRACT: It is a known fact that compounds found in various vegetables and fruits taken by diet may be of help in treating cancer. A polyphenolic compound, quercetin was reported to have a possible impact on some types of cancer through a variety of molecules. However, there are ongoing studies to prove the effect mechanisms and it has yet to be clarified. Tamoxifen is a non-steroidal anti-estrogen drug used in the treatment of estrogen-positive breast cancer. We already have some information regarding occasional use of tamoxifen in treatment of some cancers other than breast cancer, and thus we began investigating the effects of these two components on apoptosis in colon cancer cells in our study considering that it may also be effective in colon cancer. The effect of quercetin and tamoxifen on apoptotic index was evaluated by Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) method at the 24th, 48th, and 72nd hours. In colon cancer cells, the effects of various concentrations (25, 50, and 100 μM) of quercetin and tamoxifen on apoptotic index in colon cancer cells were established. Apoptotic index increased in groups that were administered quercetin and tamoxifen compared to control groups. The current study shows that quercetin and tamoxifen may play an important role in the treatment of cancer by inducing apoptosis in colon adenocarcinoma.
KEYWORD: Apoptosis, CaCo-2, Quercetin, Colon Cancer, Tamoxifen

KOLON KANSER HÜCRELERİNİN APOPTOZUNU KERSETİN VE TAMOKSİFEN NASIL ETKİLER?

ÖZET: Diyetle alınan çeşitli sebze ve meyvelerde bulunan bileşiklerin kanser tedavisine yardımcı olabileceği bilinmektedir. Polifenolik bir bileşik olan kersetinin de bazı kanserlerde çeşitli moleküller üzerinden etkili olabildiği hakkında mevcut araştırmalar vardır. Ancak etki mekanizmalarının belirlenmesi için çalışmalar devam etmekte olup, tam olarak açıklığa kavuşmamıştır. Tamoksifen ise östrojen pozitif meme kanserinin tedavisinde kullanılan bir nonsteroid anti-östrojen ilaçtır. Zaman zaman meme kanseri dışında diğer bazı kanserlerde de kullanıldığı hakkında çeşitli bilgilere sahip olduğumuz tamoksifenin, kolon kanserinde de etkili olabileceğini düşünerek araştırmamızda kolon kanser hücrelerinde bu iki bileşenin apoptoz üzerine etkilerini incelemeyi amaçladık. Kersetin ve tamoksifenin 24, 48 ve 72. saatlerde apoptotik indekse olan etkileri TUNEL metodu belirlendi. Kolon kanser hücrelerinde kersetin ve tamoksifenin çeşitli konsantrasyonlarda (25, 50 ve 100 μM) apoptotik indekse olan etkileri belirlendi. Bulgular: Apoptotik indeksin kersetin ve tamoksifen uygulanan gruplarda kontrol grubuna göre arttığı bulundu. Apoptotic index increased in groups that were administered quercetin and tamoxifen compared to control groups. Kolon adeno karsinomada kersetin ve tamoksifenin apoptozu indükleyerek kanser tedavisinde önemli bir rol oynayabileceğini bulunmuştur.
ANAHTAR KELİMELER: Apoptoz, CaCo-2, Kersetin, Kolon Kanseri, Tamoksifen

1. Introduction

Colon cancer is among the most common cause of cancer death around the world (1). It is known that the development of colon cancer is related with excessive cell proliferation and disorders observed in apoptosis. Estrogen receptors, also found in normal and colon cancer cells, have been shown to play a role on cell proliferation and apoptosis in colon carcinogenesis (2-4). Natural compounds that also include flavonoids and their active phytochemicals present chemopreventive effects through arresting cell cycle and inducing apoptosis (5). Quercetin (3,3',4',5,7-pentahydroxyflavone) is a significant flavonoid found in various vegetables, fruits, seeds, tea, and red wine taken with diet. Quercetin demonstrates pro-apoptotic effects in tumor cells and may block growth in various phases of cell cycle in a variety of human cancer cell series. Quercetin can be also modulate estrogen receptor activation (6-10). Another agent we used in our study, tamoxifen is a non-steroidal anti-estrogen drug commonly used in the treatment of estrogen receptor positive breast cancer (11, 12). Among the effect mechanisms of tamoxifen is to bind to estrogen receptor in order to prevent the recurrence of breast carcinoma and to inhibit the growth of cancer cells. Tamoxifen may be having same effect in colon carcinoma. Tamoxifen also demonstrates its effect through growth factors and immune system, and thus, it provides certain benefits for estrogen-neutral patients (11-13). The aim of our study is to investigate the effects of quercetin and tamoxifen on apoptosis in CaCo-2 human colon adenocarcinoma cell line.

2. Materials and Methods

Human colon adenocarcinoma cell line CaCo-2 was used in order to examine the effects of quercetin and tamoxifen on apoptotic index. CaCo-2 colon cancer cells supplied from American Type Culture Collection (ATCC) were grown in Eagle's minimal essential medium (Biowest, Nuaille´, France) that contained 10% fetal calf serum, penicillin-streptomycin in 37°C under 5% CO2 atmospheric pressure at the Medical Biology Laboratory of Eskisehir Osmangazi University. Tamoxifen (Sigma-Aldrich Inc., St. Louis, USA) and quercetin (Sigma-Aldrich Inc., St. Louis, MO, USA) solubilized in dimethyl sulfoxide (DMSO) were applied separately to CaCo-2 cells in 25, 50, and 100 μM concentrations. The effect of quercetin and tamoxifen on apoptotic index was established with Apop Taq Plus Peroxidase In Situ Apoptosis Detection kit (Chemicon International, Huissen, The Netherlands) at the 24th, 48th, and 72nd hours. To determine apoptotic index, apoptotic nuclei were counted at each chamber slide of cell lines culture. 500 cells of in the different 10 areas were evaluated in each slide. Apoptotic index: Number of apoptotic nuclei/total cell number x 100 (14).

Statistical Analysis

SPSS Windows 15.0 and Sigmastat 3.5 were used in analyses. In analyzing the suitability of data to normal distribution, Shapiro Wilk's test was utilized. The comparisons between the groups were performed using one way variance (ANOVA) analysis. Data were summarized as mean ± standard error. $p<0.05$ was accepted to be statistically significant.

3. Results

Quercetin was observed to increase apoptotic index at the 48th hour in 50 μM and 100 μM concentrations ($p<0.001$) and at the 72nd hour in 25 μM ($p<0.001$) and 50 μM ($p<0.05$) concentrations (Figure 1A-1B). A statistical difference was not observed in other concentrations and hours compared to the control group ($p>0.05$). Tamoxifen was found to increase apoptotic index at the 24th hour in 25 μM and 100 μM concentrations ($p<0.001$), at the 48th hour in 25 μM ($p<0.001$) and 100 μM ($p<0.01$) concentrations and at the 72nd hour in all doses ($p<0.001$) (Figure 1C-1D). A statistical difference was not observed in other concentrations and at other hours compared to the control group ($p>0.05$). Apoptotic index was observed to have the highest increase in groups that were administered quercetin 50 μM at the 24th hour, 100 μM at the 48th hour, and 25 μM at the 72nd hour, and Tamoxifen 100 μM at the 24th hour, 25 μM at the 48th hour, and 100 μM at the 72nd hour (Table I). Tamoxifen was observed to increase apoptotic index more than quercetin considering all the results.

Table 1

Apoptotic index was analyzed by TUNEL assay in CaCo-2 cell line

Groups and Doses		Apoptotic Index		
Groups	Doses (μM)	24th hour	48th hour	72nd hour
Control Group (C)	0	1.827±0.237	1.848±0.219	2.020±0.209
	25	6.153±0.956	2.306±0.202	5.741±0.662
Quercetin (Q)	50	6.516±0.792	4.361±0.488	3.749±0.374
	100	5.787±0.399	6.824±0.572	3.416±0.290
	25	4.723±0.449	5.484±0.508	5.959±0.349
Tamoxifen (T)	50	3.137±0.288	2.895±0.288	5.028±0.475
	100	6.931±0.706	3.963±0.529	5.968±0.436
Statistical Analysis		$C\text{-}Q_{25}^{ns}$	$C\text{-}Q_{25}^{n.s}$	$C\text{-}Q_{25}^{***}$
		$C\text{-}Q_{50}^{ns}$	$C\text{-}Q_{50}^{***}$	$C\text{-}Q_{50}^{*}$
		$C\text{-}Q_{100}^{ns}$	$C\text{-}Q_{100}^{***}$	$C\text{-}Q_{100}^{ns}$
		$C\text{-}T_{25}^{***}$	$C\text{-}T_{25}^{***}$	$C\text{-}T_{25}^{***}$
		$C\text{-}T_{50}^{ns}$	$C\text{-}T_{50}^{ns}$	$C\text{-}T_{50}^{***}$
		$C\text{-}T_{100}^{***}$	$C\text{-}T_{100}^{**}$	$C\text{-}T_{100}^{***}$

mean ± s.e. values are shown for three experiments (n=3) (^{ns}: not significant, *: p<0.05, **: p<0.01, ***: p<0.001).

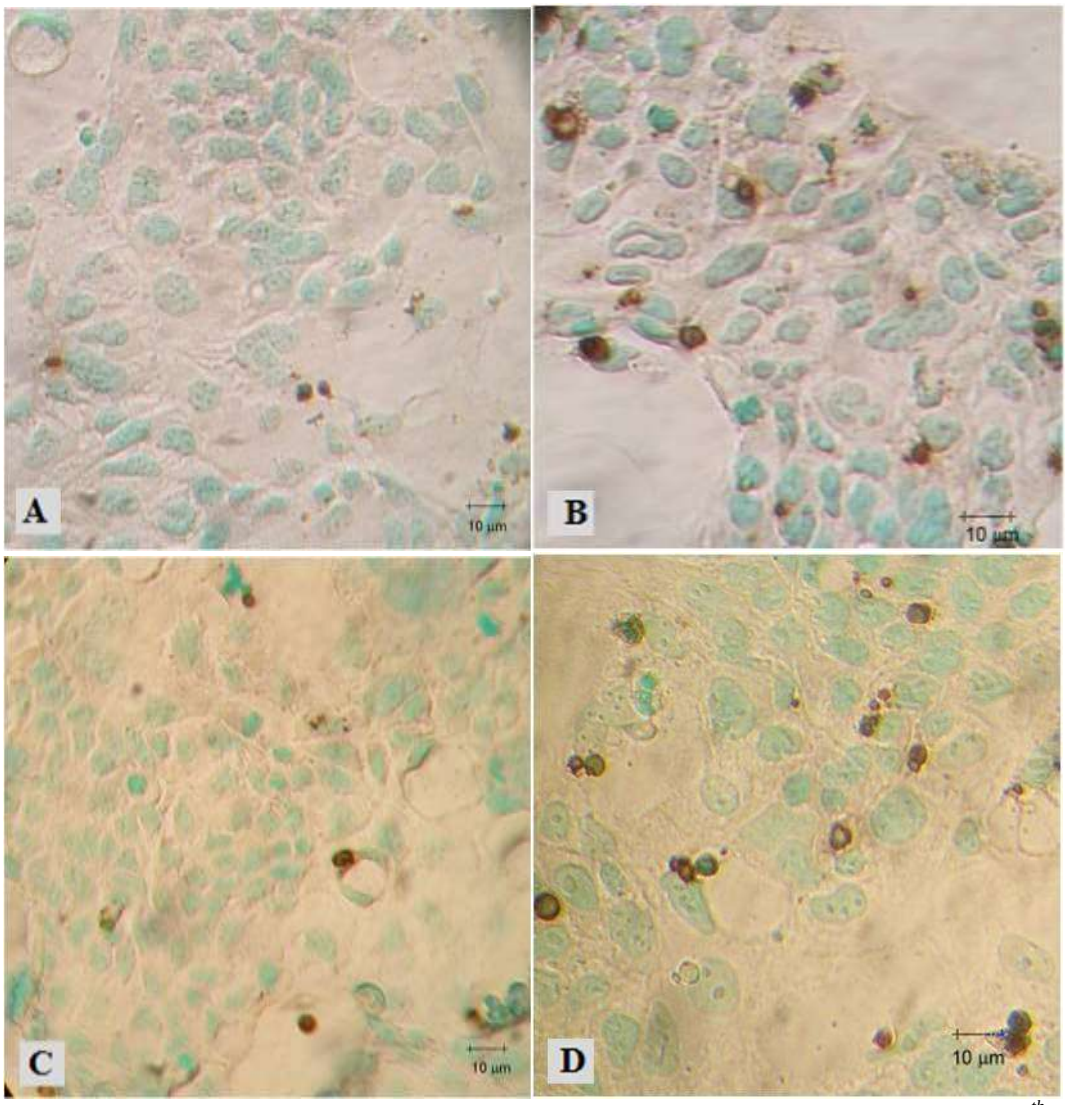

Figure 1. *Apoptosis imaged obtained via TUNEL staining in CaCo-2 cells. Control group at the 48th hour (A), group that was administered 100 μM quercetin at the 48th hour (B), control group at the 24th hour (C) and group that was administered 100 μM tamoxifen at the 24th hour (D).*

4. Discussion

The development of colon cancer is related with excessive cell proliferation and disorders observed in apoptosis. In the present study the effects of quercetin and tamoxifen were tested on apoptosis in CaCo-2 colon carcinoma cell line. Previous in vitro studies have reported that quercetin induce apoptosis and inhibits the growth in various colon adenocarcinoma cell lines. In the study it was used on colon adenocarcinoma HT-29 and SW480 cell lines, and it showed that use of 25, 50 and 100 μM quercetin for 48 or 96 hours inhibited cell growth and induced apoptosis. Investigating the effect of quercetin on the protein expression of the anti-apoptotic protein Bcl-2 and the pro-apoptotic protein Bax showed that Bcl-2 levels were reduced in a dose-dependent manner in cells treated with quercetin for 72 hours, but no change was shown in the Bax levels, suggesting that the decreased Bcl-2 may be one reason for caspase-3 being activated and the cleavage of PARP which followed (15). It was shown that quercetin reduced cell vitality depending on dose and time and induced apoptosis depending on dose on human colon adenocarcinoma cell line SW480. After SW480 cells were treated with 80 micromol/L of quercetin for 48 hours, the cells were observed to have increased in the G(2)/M phase. It has been reported that the induction of apoptosis was dose-dependent at concentrations of 20, 40, 60, and 80 micromol/L of quercetin (16). It was reported that 100 μM quercetin affected cell cycle and apoptosis-related genes and inhibited cell growth in G1/S and G2/M phases on colon adenocarcinoma CO115 cell lines (17). The mechanism of quercetin-induced apoptosis in HT-29 colon cancer cells was demonstrated in a study which showed that the viability of cells treated with quercetin decreased significantly depending on the dose. In particular, quercetin brought about an increase in cell cycle arrest in the G1 phase and up-regulated apoptosis-related proteins, such as AMPK, p53, and p21, within 48 hours. These results show that quercetin induces apoptosis through the activation of AMPK and p53-dependent apoptotic cell death in HT-29 colon cancer cells (18). In another study, Caco-2 human colon adenocarcinoma cells were exposed to 5 or 50 μM quercetin for 48 hours. CaCo-2 cells which we also used in our study that 5 μM quercetin dose down-regulated the expressions of cell cycle genes and cell growth and induced the arrest of cell cycle. In the same study, it was also shown that the administration of 50 μM quercetin reduced cell proliferation by 51.3%, also decreased the cell percentage in G1 phase, and increased the cell percentage in sub-G1 phase (6). Similar to all these studies, we demonstrated that 50 and 100 μM quercetin induced apoptosis at the 48th hour in CaCo-2 cell ($p<0.001$).

Alongside all these studies, it has been reported that cell growth was inhibited by different quercetin concentration in HCT-116 (30-100 μM) and HT-29 (80-100 μM) colon carcinoma cell line after 24-hour exposure. It has been shown that quercetin showed anti-carcinogenic activity through lowering cell proliferation at high concentrations. However, it was also observed in these cells that quercetin induced cell proliferation in a subtle but significant manner at low concentrations. Based on these results, it has been reported that quercetin had a dualistic effect on cell proliferation (19). Concentrations of quercetin used in our report increased apoptosis in Caco-2 cells for 24 hours but this increase was not statistically significant.

Researches show that quercetin may induce various morphological changes causing apoptosis and growth inhibition (20-23). Quercetin may play a role in sensitizing colon cancer cells to tumor necrosis factor–related apoptosis-inducing ligand (TRAIL) induced apoptosis (24). However we previously reported that quercetin increased the pro-apoptotic Bak protein levels in colon adenocarcinoma cell line (25). The results support that quercetin induced apoptosis may involve extrinsic (death receptor) and intrinsic (mitochondrial) pathways but further studies are needed to understand the molecular mechanism of quercetin -induced apoptosis.

Only a few studies were found on the effect of a non-steroidal drug tamoxifen that was another compound we used in current study on colon cancer. However, the effects of estrogen receptor ligands such as estradiol, 17 epiestriol, and quercetin in DLD-1 cells were investigated in a study. In the mentioned study, tamoxifen and steroid combinations were shown to inhibit the cell growth with tumor-static effect on colon cancer cells (26).

The study suggests that tamoxifen is an anti-tumor agent in colon cancer. Contrary to this study, another study reported that tamoxifen was not able to have an effect on tumor growth in colon cancer on its own (27).

In another study, the effect of tamoxifen (1 microM Tx, 5 microM Tx) on cell growth in DLD-1, CACO-2, SW-620, and COLO-205 colon cancer cells, and SW-1463 and SW-837 rectal cancer cells was investigated. It was found that tamoxifen inhibited the growth of colon cancer cells in an environment that contained no serum, but it did not inhibit the growth of rectal cancer cells (28). The same research group, in another study, investigated the effect of tamoxifen on cell growth at lower concentrations (0.005 microM, 0.05 microM, 0.5 microM, 5 microM, and 50 microM) in colorectal cancer cells. For this, while investigating HT-29 and SW-620 colon cancer cells and SW-1463 rectal cancer cells in media both with and without serum, they also investigated COLO-205 colon cancer and SW-837 rectal cancer cells only in a medium containing serum. In the non-serum containing medium, it was observed that tamoxifen inhibited growth in HT-29 and SW-620 colon cancer cells at all concentrations. While in the serum-containing medium, they reported that tamoxifen inhibited growth in SW-837 rectal cancer cells at all concentrations, and it was effective on SW-1463 rectal cancer cells at concentrations of 0.05 microM and 0.5 microM. In conclusion, it was demonstrated that tamoxifen may show different effects on growth in colon and rectal cancers under various conditions (29). In our research, tamoxifen was also seen to be effective in inducing apoptosis in colon cancer cells grown in an environment with a serum-containing medium.

In another study, the effects of estradiol and tamoxifen on growth in murine colon 38 cancer cells, applied on their own at various concentrations (10–4 to 10–12 M) or together with the chemotherapy agent fluorouracil (FU), were investigated. It was observed that tamoxifen inhibited cancer growth at a concentration of 10–4 M. It was reported that the agents used in the study increased the apoptosis of tumor cells at different concentrations and in different incubation periods. In conclusion, it was demonstrated that sex steroids played a role in colon carcinogenesis (30).

The effects of tamoxifen on cell proliferation, vigor, and apoptosis in HCT8 and HCT116 colon cancer cells at concentrations of 0.1, 1, 5, and 10 microM have been investigated. It was shown that 5 microM of tamoxifen significantly reduced growth in HCT8 cells, but was less effective in HCT116 cells. It was found that 10 microM of tamoxifen was lethal only in HCT8 cells. Cell vigor was also reduced at a concentration of 5 microM of tamoxifen only in HCT8 cells. However, it was also reported that tamoxifen concentrations did not induce apoptosis in either cell series (4).

Our study showed that tamoxifen could realize such an anti-tumor effect by inducing apoptosis in colon cancer. It was reported in another study that this in vitro estrogen-sensitive cell-specific response might partially reflect differential tumor biology for different individuals (31).

5. Conclusion

The current research showed that quercetin and tamoxifen increased apoptotic activity of colon cancer cells. We have previously reported that quercetin and tamoxifen reduced telomerase activity in colon cancer cells (32). This result supports the ones we concluded in this current study. Consequently in our study, we believe that quercetin and tamoxifen may have a potential to be used as a chemotherapeutic drug in colon carcinoma. However, further studies are needed to demonstrate such effects more in colon carcinoma.

6. Acknowledgments

This study was presented as a poster in 4th International Congress of Molecular Medicine Congress.

REFERENCES

1. Johnson, D.Y. Wadhwa, S. and Johnson, F.E. (2013). Colon and Rectum Carcinoma. In *Patient Surveillance After Cancer Treatment*: Springer. 179-183.
2. Konstantinopoulos, P. Kominea, A. Vandoros, G. Sykiotis, G. Andricopoulos, P. Varakis, I. Sotiropoulou-Bonikou, G. and Papavassiliou, A. (2003). Oestrogen receptor beta (ERβ) is abundantly expressed in normal colonic mucosa, but declines in colon adenocarcinoma paralleling the tumour's dedifferentiation. *European Journal of Cancer* 39:1251-1258.
3. Campbell-Thompson, M. Lynch, I.J. and Bhardwaj, B. (2001). Expression of estrogen receptor (ER) subtypes and ERbeta isoforms in colon cancer. *Cancer Res* 61:632-640.
4. Picariello, L. Fiorelli, G. Martineti, V. Tognarini, I. Pampaloni, B. Tonelli, F. and Brandi, M.L. (2002). Growth response of colon cancer cell lines to selective estrogen receptor modulators. *Anticancer research* 23:2419-2424.
5. Rajamanickam, S. and Agarwal, R. (2008). Natural products and colon cancer: current status and future prospects. *Drug Dev Res* 69:460-471.
6. van Erk, M.J. Roepman, P. van der Lende, T.R. Stierum, R.H. Aarts, J. van Bladeren, P.J. and van Ommen, B. (2005). Integrated assessment by multiple gene expression analysis of quercetin bioactivity on anticancer–related mechanisms in colon cancer cells in vitro. *European journal of nutrition* 44:143-156.
7. Galluzzo, P. Martini, C. Bulzomi, P. Leone, S. Bolli, A. Pallottini, V. and Marino, M. (2009). Quercetin-induced apoptotic cascade in cancer cells: Antioxidant versus estrogen receptor α-dependent mechanisms. *Molecular nutrition & food research* 53:699-708.
8. Gibellini, L. Pinti, M. Nasi, M. Montagna, J.P. De Biasi, S. Roat, E. Bertoncelli, L. Cooper, E.L. and Cossarizza, A. (2011). Quercetin and cancer chemoprevention. *Evidence-Based Complementary and Alternative Medicine* 2011.
9. Bulzomi, P. Galluzzo, P. Bolli, A. Leone, S. Acconcia, F. and Marino, M. (2012). The pro-apoptotic effect of quercetin in cancer cell lines requires ERβ-dependent signals. *Journal of Cellular Physiology* 227:1891-1898.
10. Kumar, R. Verma, V. Jain, A. Jain, R.K. Maikhuri, J.P. and Gupta, G. (2011). Synergistic chemoprotective mechanisms of dietary phytoestrogens in a select combination against prostate cancer. *J Nutr Biochem* 22:723-731.
11. Khalkhali-Ellis, Z. Christian, A.L. Kirschmann, D.A. Edwards, E.M. Rezaie-Thompson, M. Vasef, M.A. Gruman, L.M. Seftor, R.E. Norwood, L.E. and Hendrix, M.J. (2004). Regulating the Tumor Suppressor Gene Maspin in Breast Cancer Cells A Potential Mechanism for the Anticancer Properties of Tamoxifen. *Clinical cancer research* 10:449-454.
12. Petinari, L. Kohn, L.K. de Carvalho, J.E. and Genari, S.C. (2004). Cytotoxicity of tamoxifen in normal and tumoral cell lines and its ability to induce cellular transformation in vitro. *Cell biology international* 28:531-539.
13. Aldous, W.K. Marean, A.J. DeHart, M.J. Matej, L.A. and Moore, K.H. (1999). Effects of tamoxifen on telomerase activity in breast carcinoma cell lines. *Cancer* 85:1523-1529.
14. Cosan, D. Soyocak, A. Basaran, A. Degirmenci, I. and Güneş, H.V. (2009). The effects of resveratrol and tannic acid on apoptosis in colon adenocarcinoma cell line. *Saudi Med J* 30:191-195.
15. Kim, W.K. Bang, M.H. Kim, E.S. Kang, N.E. Jung, K.C. Cho, H.J. and Park, J.H. (2005). Quercetin decreases the expression of ErbB2 and ErbB3 proteins in HT-29 human colon cancer cells. *J Nutr Biochem* 16:155-162.
16. Shan, B.-E. Wang, M.-X. and Li, R.-q. (2009). Quercetin inhibit human SW480 colon cancer growth in association with inhibition of cyclin D1 and survivin expression through Wnt/β-catenin signaling pathway. *Cancer investigation* 27:604-612.
17. Murtaza, I. Marra, G. Schlapbach, R. Patrignani, A. Künzli, M. Wagner, U. Sabates, J. and Dutt, A. (2006). A preliminary investigation demonstrating the effect of quercetin on the expression of genes related to cell-cycle arrest, apoptosis and xenobiotic metabolism in human CO115 colon-adenocarcinoma cells using DNA microarray. *Biotechnology and applied biochemistry* 45:29-36.
18. Kim, H.J. Kim, S.K. Kim, B.S. Lee, S.H. Park, Y.S. Park, B.K. Kim, S.J. Kim, J. Choi, C. Kim, J.S. et al. (2010). Apoptotic effect of quercetin on HT-29 colon cancer cells via the AMPK signaling pathway. *J Agric Food Chem* 58:8643-8650.
19. van der Woude, H. Gliszczyńska-Świgło, A. Struijs, K. Smeets, A. Alink, G.M. and Rietjens, I.M. (2003). Biphasic modulation of cell proliferation by quercetin at concentrations physiologically relevant in humans. *Cancer letters* 200:41-47.
20. Ak, A. Başaran, A. Dikmen, M. Coşan, D.T. Değirmenci, İ. and Güneş, H.V. (2011).

Evaluation of Effects of Quercetin (3, 3', 4', 5, 7-pentohidroxyflavon) on Apoptosis and Telomerase Enzyme Activity in MCF-7 and NIH-3T3 Cell Lines Compared with Tamoxifen. *Balkan Medical Journal* 28:293-299.

21. Choi, J.A. Kim, J.Y. Lee, J.Y. Kang, C.M. Kwon, H.J. Yoo, Y.D. Kim, T.W. Lee, Y.S. and Lee, S.J. (2001). Induction of cell cycle arrest and apoptosis in human breast cancer cells by quercetin. *Int J Oncol* 19:837-844.
22. Nichenametla, S.N. Taruscio, T.G. Barney, D.L. and Exon, J.H. (2006). A review of the effects and mechanisms of polyphenolics in cancer. *Critical reviews in food science and nutrition* 46:161-183.
23. Rodgers, E.H. and Grant, M.H. (1998). The effect of the flavonoids, quercetin, myricetin and epicatechin on the growth and enzyme activities of MCF7 human breast cancer cells. *Chemico-biological interactions* 116:213-228.
24. Psahoulia, F.H. Drosopoulos, K.G. Doubravska, L. Andera, L. and Pintzas, A. (2007). Quercetin enhances TRAIL-mediated apoptosis in colon cancer cells by inducing the accumulation of death receptors in lipid rafts. *Molecular cancer therapeutics* 6:2591-2599.
25. Soyocak, A. Turgut Coşan, D. Başaran, A. Değirmenci, İ. and Güneş, H.V. (2009). The Association Between Apoptotic Bak Protein And Quercetin in Breast and Colon Cancer Cell Lines. *FABAD J. Pharm. Sci* 34:83-89.
26. Nakayama, Y. Sakamoto, H. Satoh, K. and Yamamoto, T. (2000). Tamoxifen and gonadal steroids inhibit colon cancer growth in association with inhibition of thymidylate synthase, survivin and telomerase expression through estrogen receptor beta mediated system. *Cancer letters* 161:63-71.
27. Shen, L.-Z. Hua, Y.-B. Yu, X.-M. Xu, Q. Chen, T. Wang, J.-H. and Wu, W.-X. (2005). Tamoxifen can reverse multidrug resistance of colorectal carcinoma in vivo. *World J Gastroenterol* 11:1060-1064.
28. Ziv, Y. Gupta, M. Milsom, J. Vladisavljevic, A. Brand, M. and Fazio, V. (1993). The effect of tamoxifen and fenretinimide on human colorectal cancer cell lines in vitro. *Anticancer research* 14:2005-2009.
29. Ziv, Y. Gupta, M. Milsom, J. Vladisavljevic, A. Kitago, K. and Fazio, V. (1995). The effect of tamoxifen on established human colorectal cancer cell lines in vitro. *Anticancer research* 16:3767-3771.
30. Motylewska, E. Ławnicka, H. and Mełeń-Mucha, G. (2007). Oestradiol and tamoxifen inhibit murine Colon 38 cancer growth and increase the cytotoxic effect of fluorouracil. *Endokrynologia Polska* 58:426-434.
31. Leo, A. Messa, C. Cavallini, A. and Linsalata, M. (2001). Estrogens and colorectal cancer. *Current Drug Targets-Immune, Endocrine & Metabolic Disorders* 1:1-12.
32. Cosan, D.T. Soyocak, A. Basaran, A. Degirmenci, İ. Gunes, H.V. and Sahin, F.M. (2011). Effects of various agents on DNA fragmentation and telomerase enzyme activities in adenocarcinoma cell lines. *Molecular biology reports* 38:2463-2469.
.