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Investigation of the effects of Protocatechuic acid on apoptosis, oxidant and antioxidant status in Caco-2 colorectal cancer cells

Protokatekuik asidin Caco-2 kolorektal kanser hücrelerinde apoptoz, oksidan ve antioksidan durum üzerine etkilerinin araştırılması

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ABSTRACT

Aim: Protocatechuic acid (PCA) is one of the common phenolic acids found in many foods and plants and it has multiple biological activities. Although PCA has been investigated for its antioxidant, anti-inflammatory, and anticancer effects in various cell lines, its effects on molecules involved in the apoptotic pathway, especially in human colon cancer (Caco-2) cells, have not been fully elucidated. This study aimed to investigate the effects of PCA on possible oxidant, antioxidant, and apoptosis mechanisms in Caco-2 cells, depending on dose and time.
Methods: In the experimental study, 4 groups were created: control (K), PCA (250-500-1000 µM). Total oxidant capacity (TOC), total antioxidant capacity (TAC), Oxidative stress index (OSI), Bax, Bad, Bcl-2, Bcl-xl, and Caspase 9 protein levels were determined by the ELISA method in the cell lysates obtained from the groups.
Results: The results showed that PCA treatment had apoptotic effects on Caco-2 cells at 24 and 48 h. PCA also decreased OSI levels by increasing TAC levels and decreasing TOC levels in a dose-dependent manner.
Conclusion: As a result, it was determined that PCA has an apoptotic effect on the Caco-2 cell line and can be useful in the prevention and/or treatment of colon cancer.

Keywords: Colon cancer, Caco-2, Protocatechuic acid, Apoptosis, Oxidative stress

ÖZ

Amaç: Protokatekuik asit (PCA), birçok biyolojik aktiviteye sahip birçok gıda ve bitkide bulunan yaygın fenolik asitlerden biridir. PCA'nın çeşitli hücre hatlarında antiinflamatuvar, antioksidan, antikanser etkileri araştırılmasına rağmen insan kolon kanseri (Caco-2) hücrelerinde özellikle de apoptotik yolaklakta yer alan moleküller üzerindeki etkileri tam olarak aydınlatılamamıştır. Bu çalışmanın amacı PCA'nın Caco-2 hücrelerinde doza ve zamana bağlı olarak olası oksidan, antioksidan ve apoptoz mekanizmaları üzerindeki etkilerini araştırmaktır.
Yöntem: Deneysel çalışmada kontrol (K), PCA (250-500-1000 µM) olmak üzere 4 grup oluşturulmuştur. Gruplardan elde edilen hücre lizatlarında toplam oksidan kapasite (TOC), toplam antioksidan kapasite (TAC), Oksidatif stres indeksi (OSI), Bax, Bad, Bcl-2, Bcl-xl, Kaspaz 9 protein seviyeleri ELISA yöntemiyle belirlenmiştir.
Bulgular: Sonuçlar PCA tedavisinin Caco-2 hücrelerinde 24 ve 48 saatte apoptotik etkilere sahip olduğunu göstermiştir. Ayrıca PCA doza bağlı bir şekilde TAC seviyelerini artırırken TOC seviyelerini azaltarak OSI seviyelerini de azaltmıştır.
Sonuç: Sonuç olarak PCA'nın Caco-2 hücre hattında apoptotik etkiye sahip olduğu ve kolon kanserinin önlenmesinde ve/veya tedavisinde yararlı olabileceği belirlenmiştir.

Anahtar kelimeler: Kolon kanseri, Caco-2, Protokatekuik asit, Apoptoz, Oksidatif stres

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Introduction

Colon cancer is the third most common type of cancer in the world, starting in the large intestine and spreading to the lower digestive system [1, 2]. Various surgical methods and chemotherapy drugs are used in the treatment of the disease. However, the side effects of these drugs are high, and the cancer cells become resistant to the drugs used over time, which negatively affects the treatment process [3]. Therefore, there is a need to develop new drug candidates that will minimize the side effects. Phenolic compounds with antioxidant activity especially stand out in controlling this disease [4-6]. Flavonoids and phenolic compounds have a strong cytotoxic effect against colon cancer cells with lower risk and fewer side effects [7]. In addition, metabolites such as flavonoids and phenolic compounds can reduce tumor cell proliferation through various mechanisms, such as activating caspases and promoting apoptosis [2]. Protocatechuic acid (3,4-dihydroxybenzoic acid, PCA), among the phenolic compounds, may be a useful agent with its remarkable antioxidant activity [8,9]. Various studies have reported that PCA has antioxidant, antibacterial, antimutagenic, antiviral, anti-inflammatory, antiulcer, antidiabetic, neuroprotective, and anticancer properties [10,11,12]. Although there are many studies on PCA in various types of cancer, studies on colon cancer cell lines are limited.

It is well known that apoptosis is one of the main pathways of tumor cell death. According to literature reviews, PCA is considered a potential chemopreventive compound for clinical applications in the prevention of neoplastic diseases and a highly promising compound in the treatment of various diseases. However, the mechanism of action of PCA on apoptotic pathways, where it disrupts specific pathways leading to cell death, has not yet been elucidated. Therefore, further research is needed in this area. The mechanism of apoptosis is complex and involves many signaling pathways. Apoptosis activation is initiated by two main pathways: intrinsic (mitochondria-dependent) extrinsic. Both pathways combine to activate caspases and ultimately cause morphological and biochemical cellular changes, leading to the onset of apoptosis [13,14]. The intrinsic pathway is regulated by Bcl-2 (B cell lymphoma-2) family members [(anti-apoptotic (Bcl-2, Bcl-xl)- pro-apoptotic (Bad, Bax)]. Pro-apoptotic proteins such as Bcl-2 Bax (Bcl-2-associated X) and Bad (Bcl-2-associated death) activate caspases, releasing the mitochondrial intermembrane space and promoting cell death [15]. In addition, members such as Bcl-2 inhibit cell death by suppressing cytochrome c release. Therefore, the balance between pro- and antiapoptotic proteins is important in determining apoptosis. In summary, apoptosis is a promising target in tumor therapy.

In light of this information, this study investigated the effects of PCA on possible oxidant, antioxidant, and apoptosis mechanisms in Caco-2 cells, depending on dose and time. I believe that in the future, PCA will contribute to developing new candidate anticancer agents by elucidating the signaling pathways associated with apoptosis mechanisms.

Materials and Methods

Cell Culture and Experimental Groups

PCA was purchased from Sigma Aldrich (Cat No. 99-50-3). The human colon cancer cell line (Caco-2) was used from cells available in our stock. Caco-2 cells were cultured in DMEM medium containing 10% FBS (fetal bovine serum), L-glutamine, 1% penicillin-streptomycin, and NaHCO3 at 37oC in an environment containing 5% CO2 and atmospheric humidity. Incubated at 37°C and passaged every 2-3 days. PCA was dissolved in ethyl alcohol, and the concentration of ethyl alcohol was less than 0.1% for all treatments. The PCA concentrations used in the study were determined based on the results of our previous study [16].

In this study, 4 main groups were created as follows.

Group I (Control): Cells in this group were not treated with any chemicals.

Group II (250 µM PCA): Cells were incubated with PCA (250 µM) for 24 and 48 h [16].

Group III (500 µM PCA): Cells were incubated with PCA (500 µM) for 24 and 48 h [16].

Group IV (1000 µM PCA): Cells were incubated

with PCA (1000 µM) for 24 and 48 h [16].

Detection of apoptosis

The apoptotic markers in the PCA-treated or untreated control groups were determined using the ELISA method. According to the manufacturer's instructions, the Bad, Bax, Bcl-2, and Caspase 9 protein levels in Caco-2 cell culture lysates were determined using commercial ELISA assay kits (BT-LAB ELISA kits, China). The results were obtained by taking OD values at a wavelength of 405 nm with an ELISA microplate reader (BIOTEK ELx808, USA).

Determination of TAC, TOC, and OSI

To evaluate antioxidant and oxidative stress levels, TOC, TAC, and levels were analyzed in line with the instructions of the manufacturer company using ELISA kits (RL0017 Rel Assay Total Antioxidant Status, RL0024 Rel Assay Total Oxidant Status). Studies were performed using an ELISA reader and an ELISA plate washer. TAC findings were expressed as mmol Trolox Equiv/L, while TOC findings were expressed as mmol H2O2 Equiv/L [17]. In addition, the OSI was calculated using TOC and TAC measurements [18].

Statistical analyses

All data were expressed as mean ± standard deviation (SD). The “SPSS 20.0 for Windows” package program and the “One Way ANOVA-Tukey” test were used to evaluate the data obtained in our study. Differences between the groups were tested by Tukey’s multiple comparison post hoc tests, and p<0.05 was determined to be statistically significant. Other statistical analyses between experimental groups were calculated using GraphPad Prism (Version 7.04 for Windows, USA) software.

Results:

Results of Apoptosis

The effect of PCA on molecules involved in the apoptotic pathway is shown in Figure 1. When the study results regarding Bad protein were evaluated, a statistically significant decrease was observed in the concentrations applied to PCA in Caco-2 cells at 250 µM and 1000 µM after 24 h compared to the control group (p <0.01). After 48 h, a significant increase was observed only in the group applied with 1000 µM PCA compared to the control group (p <0.05) (Figure 1A).

Bax/Bcl-2 ratio showed a significant increase in Caco-2 cells exposed to PCA at a concentration of 250 µM for 24 h compared to the control group (p<0.01), while this increase at other doses was not statistically significant. After 48 h of application, the Bax/Bcl-2 ratio showed a significant decrease only at the concentration of 1000 µM (p<0.05) (Figure 1B). Bax/Bcl-xl ratio showed a statistically significant decrease at all concentrations applied at the end of the 24 h compared to the control group (p<0.05, p<0.01). At the end of the 48 h, a significant decrease was observed only at the concentration of 1000 µM (p<0.01) compared to the control group (Figure 1C). Caco-2 cells treated with PCA for 24 h, showed a significant increase in caspase 9 levels at all concentrations applied compared to the control group (p<0.001). At the end of the 48 h, it was observed that the increase in caspase 9 protein level continued significantly in PCA-applied Caco-2 cells compared to the control group, depending on the increasing dose concentration (p<0.001, p<0.0001). (Figure 1D).

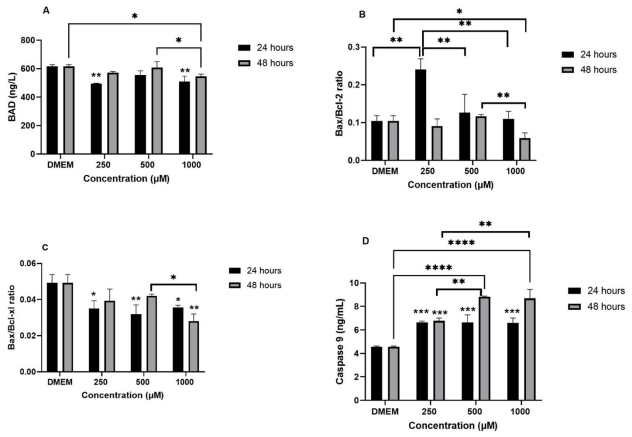

Figure 1. The effect of PCA treatment on Bad protein (A), Bax/Bcl-2 (B), Bax/Bcl-xl ratio (C), Caspase 9 protein (D) levels (mean ± SD). *P<0.05 **P<0.01, ***P<0.001, ****P<0.0001 when compared with control and other groups (n=3)

Results of TAC, TOC, and OSI

It was determined that TAC levels in Caco-2 cells applied with PCA at concentrations of 250, 500, and 1000 µM for 24 h increased significantly compared to the control group (p<0.0001). Similarly, it was

found that the increase in TAC levels continued in all treatment groups after 48 h compared to the control group ($p<0.0001$) (Figure 2A). In Caco-2 cells, a decrease in TOC levels was detected in the groups applied with PCA at concentrations of 250, 500, and 1000 µM for 24 h compared to the control group, but the decrease was significant only at the dose of 500 µM compared to the control group ($p<0.05$). In 48 h PCA applications, TOC levels decreased at doses of 250 and 500 µM compared to the control group, while TOC levels increased significantly at the dose of 1000 µM compared to the control group ($p<0.01$) (Figure 2B). When OSI values were examined at the end of the 24 and 48-h period, a significant decrease was detected in all PCA-applied groups compared to the control group ($p<0.0001$) (Figure 2C).

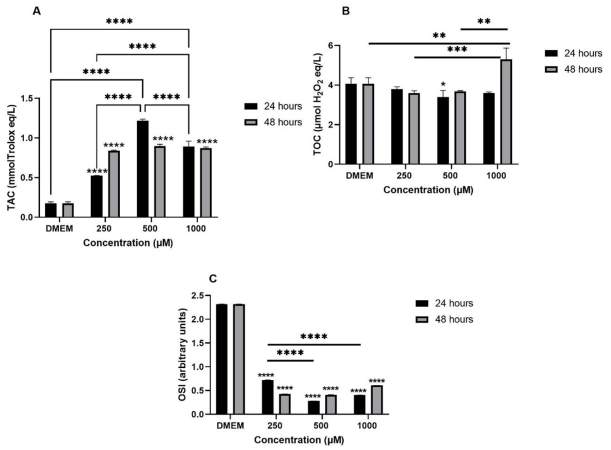

Figure 2. Mean ± standard error plot of the effects of PCA treatment on Caco-2 cells TAC (A), TOC (B), and OSI (C) **p<0.01, ***p<0.001, ****p<0.0001 when compared with control and other groups (n=3)

Discussion

Colon cancer death rates are increasing every year in the world. The apoptotic mechanism in colon cancer is a potential path that is being investigated to predict aggressive behavior. Many studies have reported the PCA anti-inflammatory, antioxidant, and anti-cancer activities in different cell lines [13]; however, very few research studies have revealed potential apoptosis mechanisms. Studies have shown that PCA protects against cell death forms such as apoptosis and pyroptosis in other types of cancer [19, 20]. Therefore, our study aimed to elucidate the effect of PCA on apoptotic pathways in the Caco-2 cell line. There are limited studies in the literature explaining the effect of PCA on Caco-2 cells through apoptosis mechanisms [16, 19].

To determine the apoptotic effect, Bcl-xl and Bcl-2 protein levels from the anti-apoptotic protein group were examined, and to determine the pro-apoptotic effect, Bax and Bad protein levels were examined. Apart from these, since caspases also carry out the apoptotic process, the caspase 9 protein level was examined.

In addition, the TAC and TOC activities of PCA were also evaluated. The suppressive effects of different doses of PCA on cell proliferation and IC50 dose after 24 and 48 h were shown in our previous study by the 3-(4,5-Dimethyltriazol-2-yl)-2,5 diphenyltetrazolium bromide (MTT) method [16]. Based on these results, the doses used in the study were decided. Today, apoptosis is the main goal of many treatment strategies and, therefore, plays an important role in the treatment of colon cancer. Bad protein is one of the pro-apoptotic proteins involved in apoptosis and triggers apoptosis via the internal pathway in the cell [21]. When the study results are considered, the concentrations applied to Caco-2 cells at 250 µM and 1000 µM PCA showed a significant decrease in 24 h compared to the control group. While there was a statistically insignificant decrease in 250 and 500 µM doses at 48 h, especially the 1000 µM dose significantly reduced Bad levels compared to the control group. These results made us think the applied doses could not have a pro-apoptotic effect.

The most studied ratio in the evaluation of apoptosis is the pro-apoptotic/anti-apoptotic (Bax/Bcl-2 or Bax/Bcl-xL) protein ratio. The imbalance between these two protein levels is considered an important indicator of apoptosis because it leads to apoptosis [22]. The Bax/Bcl-2 ratio of a cell responding to an apoptotic signal is high. This ratio was significantly increased in Caco-2 cells exposed to PCA at 250 µM concentrations for 24 h compared to the control group. The increase at 250 µM concentration in 24 h is particularly striking. After 48 h, the highest Bax/Bcl-2 ratio was observed at 500 µM concentration, but this increase was insignificant compared to the control group. These increases show us that PCA is induced in the intrinsic apoptosis pathway in Caco-

2 cells, especially at a concentration of 250 µM for 24 h, and increases sensitivity to apoptosis. When the effect of PCA on Caco-2 cells was evaluated after 48 h, it was determined that there was no statistically significant change in the Bax/Bcl-2 ratio. This is because the concentration of PCA and the incubation period may not be sufficient to increase the Bax/Bcl-2 ratio in Caco-2 cells. In this study, the Bax/Bcl-xl ratio significantly decreased in Caco-2 cells at all doses applied compared to the control group at the end of the 24 h, while it significantly decreased only at the dose of 1000 µM applied at 48 h compared to the control group. We can say that this dose has an anti-apoptotic effect. In other words, we can say that PCA reduces the Bax/Bcl-xl ratio in Caco-2 cells depending on time and dose, making the internal pathway more resistant to apoptosis. When we look at the existing studies, it has been determined that PCA has different effects on cell protection depending on the type of cancer, the time, and the concentration applied. While a study showed that high PCA concentrations induced apoptosis in Caco-2 cells [19], another study showed that it induced apoptosis at low concentrations [23].

Induction of cell death in mammalian cells in both intrinsic and extrinsic apoptotic pathways is associated with the activation of caspases. Therefore, caspase-9 protein levels were examined to determine which apoptotic pathway PCA was used in this study. Caspase-9, which is effective in the intrinsic pathway, showed a significant increase in Caco-2 cells at all concentrations applied at 24 and 48 h compared to the control group. Thus, it was determined that PCA caused caspase-9 activation in Caco-2 cells and used the intrinsic pathway. When the studies were examined, it was seen that PCA showed a significant ability to positively regulate the Bax and caspase-mediated death signaling cascade. Conversely, it created an environment that helped apoptosis induction in cancer cells by interfering with the activity of the Bcl-2 family [11]. Tsui-Hwa Tseng et al. reported that PCA showed a dose- and time-dependent inhibitory effect on human promyelocytic leukemia cell (HL-60) survival. This effect was achieved by increasing the expression of Bax, a key protein regulating apoptotic processes, while decreasing the expression of Bcl-2 [9]. In another study, it was shown that PCA, at concentrations of 1-10 µg/mL, depending on the dose used, activated the intrinsic apoptosis pathway by upregulating Bax and caspase-9, and the extrinsic apoptosis pathway by regulating caspase-8 [24]. Free radicals, oxidative stress, and antioxidants are widely studied topics today. In our study, it was determined that PCA caused an increase in TAC levels in Caco-2 cells at 24 and 48 h, depending on the increasing dose. The highest TAC values were observed at the applied concentration of 500 µM. TOC levels decreased in Caco-2 cells at 24 h compared to the control group only at the 500 µM applied concentration. While the decrease continued at 250 and 500 µM applied in Caco-2 cells at 48 h, the highest TOC values were seen in the group applied at 1000 µM. As a result, we can say that PCA significantly increased TAC levels in Caco-2 cells at 24 and 48 h compared to the control group. According to the obtained data, PCA suppressed oxidative stress by inducing antioxidant capacity in the Caco-2 cell line depending on the increasing dose concentration. In a study similar to our results, it was shown that PCA could oxidative stress, and improve the activity of antioxidant enzymes by reducing ROS levels, thus preventing oxidative damage in colon tissues [25].

Limitation: This study investigated the effect of PCA on apoptotic and antioxidant mechanisms on the Caco-2 colon cancer cell line. In addition, studies examining signaling pathways at the molecular level are needed to better understand how PCA affects the molecular mechanisms of Bax, Bcl-2, Bad genes.

Conclusion: According to our results, PCA exhibited apoptotic effects on the Caco-2 cell line depending on the dose and time. However, the mechanisms by which PCA triggers apoptosis in Caco-2 cells should be investigated in more detail at the advanced gene level with different signaling pathways and mechanisms.

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REFERENCES

1. Keum N, Giovannucci E. Global burden of colorectal cancer: emerging trends, risk factors and prevention strategies. Nat Rev Gastroenterol Hepatol. 2019;16(12):713-32. doi:10.1038/s41575-019-0189-8.
2. Esmeeta A, Adhikary S, Dharshnaa V, Swarnamughi P, Maqsummiya ZU, Banerjee A et al. Plant-derived bioactive compounds in colon cancer treatment: An updated review. Biomed Pharmacother. 2022;153,113384. doi: 10.1016/j.biopha.2022.113384.
3. Bayram D. Investigation of Appoptotic Effect of Silibinin on CCL-228-SW 480 Column Cancer Cell Line: An In Vitro Study. Süleyman Demirel University Journal of Health Sciences, 2017;8(2):1-7. doi: 10.22312/sdusbed.287465.
4. Secme M, Mutlu D, Elmas L, Arslan S. Assessing effects of caffeic acid on cytotoxicity, apoptosis, invasion, GST enzyme activity, oxidant, antioxidant status and micro-RNA expressions in HCT116 colorectal cancer cells. S Afr J Bot. 2023;157:19-26. doi: 10.1016/j.sajb.2023.03.046.
5. Kasprzak-Drozd K, Oniszczuk T, Stasiak M, Oniszczuk A. Beneficial effects of phenolic compounds on gut microbiota and metabolic syndrome. Int J Mol Sci. 2021;22(7):3715. doi: 10.3390/ijms22073715.
6. Li F, Qasim S, Li D, Dou QP. Updated review on green tea polyphenol epigallocatechin-3-gallate as a cancer epigenetic regulator. Semin Cancer Biol. 2022;83;335-52. doi: 10.1016/j.semcancer.2020.11.018.
7. Gupta, J, Ahuja A, Gupta R. Green approaches for cancers management: An effective tool for health care. AntiCancer Agents Med Chem. 2022;22(1):101-14. doi: 10.2174/1871520621666210119091826.
8. Kakkar S, Bais SA. Review on Protocatechuic Acid and Its Pharmacological Potential. ISRN Pharmacol. 2014;2014:952943. doi: 10.1155/2014/952943
9. Cadena-Iñiguez J, Santiago-Osorio E, Sánchez-Flores N, Salazar-Aguilar S, Soto-Hernández RM, Riviello-Flores MDLL et al. The Cancer-Protective Potential of Protocatechuic Acid: A Narrative Review. Molecules. 2024;29(7):1439. doi: 10.3390/molecules29071439.
10. Yang X, Sun X, Zhou F, Xiao S, Zhong L, Hu S et al. Protocatechuic acid alleviates dextran-sulfate-sodium-induced ulcerative colitis in mice via the regulation of intestinal flora and ferroptosis. Molecules 2023;28(9):3775. doi: 10.3390/molecules28093775.
11. Ya F, Li K, Chen H, Tian Z, Fan D, Shi Y et al. Protocatechuic Acid Protects Platelets from Apoptosis via Inhibiting Oxidative Stress-Mediated PI3K/Akt/GSK3β Signaling. Thromb Haemost. 2021; 121: 931–43. doi: 10.1055/s-0040-1722621.
12. AbouAitah K, Piotrowska U, Wojnarowicz J, Swiderska-Sroda A, El-Desoky AHH, Lojkowski W. Enhanced Activity and Sustained Release of Protocatechuic Acid, a Natural Antibacterial Agent, from Hybrid Nanoformulations with Zinc Oxide Nanoparticles. Int J Mol Sci. 2021;22(10):5287. doi: 10.3390/ijms22105287.
13. Okuyan D. Apoptosis. Selçuk University Faculty of Science Journal of Science, 2023;49(1):1-10. doi: 10.35238/sufefd.1210651.
14. Samsunlu ET, Lenger ÖF. Apoptotic Effects of Phytochemicals on Cancer Cells. Osmaniye Korkut Ata University Journal of The Institute of Science and Technology. 2023;6(2):1662-74.
15. Navazani P, Vaseghi S, Hashemi M, Shafaati MR, Nasehi M. Effects of Treadmill Exercise on the Expression Level of BAX, BAD, BCL-2, BCL-XL, TFAM, and PGC-1α in the Hippocampus of Thimerosal-Treated Rats. Neurotox Res. 2021;39(4):1274-84. doi: 10.1007/s12640-021-00370-w.
16. Yıldız F, Eciroglu H, Övey İS, Avnioğlu S. Effect of combination treatment of protocatechuic acid with 5-fluorouracil and oxaliplatin on colon cancer Caco-2 cell line. IJEB. 2023;61 (3):151-8. doi: 10.56042/ijeb.v61i03.65610.
17. Erel O. A new automated colorimetric method for measuring total oxidant status. Clin Biochem. 2005;38(12):1103-11. doi: 10.1016/j.clinbiochem.2005.08.008.
18. Deska M, Romuk E, Segiet OA, Bula G, Truchanowski W, Stolecka D et al. Oxidative stress and angiogenesis in primary hyperparathyroidism. Eur Surg. 2017;49(3):118-26. doi: 10.1007/s10353-016-0457-6.
19. Acquaviva R, Tomasello B, Di Giacomo C, Santangelo R, La Mantia A, Naletova I et al. Protocatechuic Acid, a Simple Plant Secondary Metabolite, Induced Apoptosis by Promoting Oxidative Stress through HO-1 Downregulation and P21 Upregulation in Colon Cancer Cells. Biomolecules. 2021;11(10):1485. doi: 10.3390/biom11101485.
20. Xiao K, Zhou M, Lv Q, He P, Qin X, Wang D et al. Protocatechuic Acid and Quercetin Attenuate ETEC-Caused IPEC-1 Cell Inflammation and Injury Associated with Inhibition of Necroptosis and Pyroptosis Signaling Pathways. J Anim Sci Biotechnol. 2023;14(1):5. doi: 10.1186/s40104-022-00816-x.
21. Elmore S. Apoptosis: a review of programmed cell death. Toxicol Pathol. 2007;35(4):495-516. doi:10.1080/01926230701320337.
22. Huang J, Zhu X, Wang H, Han S, Liu L, Xie Y et al. Role of gambogic acid and NaI (131) in A549/DDP cells. Oncol Lett. 2017;13(1):37–44. doi: 10.3892/ol.2016.5435
23. Lee WJ, Lee SH. Protocatechuic Acid Protects Hepatocytes against Hydrogen Peroxide-Induced Oxidative Stress. Curr Res Food Sci. 2022;5:222–27. doi: 10.1016/j.crfs.2022.01.006.
24. Jaganathan SK, Mandal M. Honey constituents and its apoptotic effect in colon cancer cells. JAAS. 2009;1(2):29-36. doi: 10.3896/IBRA.4.01.2.02.
25. Farombi EO, Adedara IA, Awoyemi OV, Njoku CR, Micah GO, Esogwa CU et al. Dietary Protocatechuic Acid Ameliorates Dextran Sulphate Sodium-Induced Ulcerative Colitis and Hepatotoxicity in Rats. Food Funct. 2016; 7: 913–21. doi: 10.1039/C5FO01228G.