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ORIGINAL CONTRIBUTION

Protective effects of olive oil phenolics and gallic acid on hydrogen peroxide-induced apoptosis

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Abstract
Purpose Olive oil contains several phenolic compounds possessing antioxidant activity. The aim of this study was to investigate the protective effects of olive oil phenolic extract (OOPE) and one of its constituents, gallic acid (GA) against H_2O_2-induced oxidative stress and apoptotic cell death in HeLa cells, a model for human epithelial cells.
Methods The cells were pretreated with nontoxic doses of OOPE or GA for 4, 24 and 48 h, and the intracellular reactive oxygen species (ROS) level was determined, before and after oxidative stress induction with H_2O_2. As an indicator of apoptosis, caspase 9 activity was measured.
Results All pretreatments reduced ROS generation. Four hour incubation with OOPE or GA completely inhibited ROS generation. Increases in caspase 9 activity by OOPE and GA pretreatment under harsh stress conditions were inhibited 92 and 67.8%, respectively.
Conclusions These results suggest that OOPE and GA act as powerful antioxidants against oxidative stress and exert anti-apoptotic effects.

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Keywords Anti-apoptotic effect · Gallic acid · Olive · Oxidative stress · Phenolic compounds

Introduction

Reactive oxygen species (ROS) can originate from several internal and external sources, such as oxidation–reduction reactions of normal metabolism, dietary intake of oxidative substances or cigarette smoking in humans. ROS induce a number of molecular alterations in cellular components, including proteins, lipids and DNA, lead to changes in cell morphology and viability [1]. The cells are naturally provided with an extensive array of protective enzymatic and nonenzymatic antioxidants that counteract these potentially injurious oxidizing agents [1]. However, antioxidant defense mechanisms cannot completely cope with the harmful effects of oxidative stress and oxidatively damaged molecules accumulate in the cells. Studies have shown that ROS play an important role in aging, age-related degenerative diseases, brain dysfunction, coronary heart diseases and carcinogenesis [2, 3].

Virgin olive oil contributes the health-promoting properties of the Mediterranean diet, along with fresh vegetables and fruits. Although the beneficial health effects of olive oil have been mainly attributed to its high content of oleic acid, recently greater attention has focused on minor components such as phenolic compounds, which have strong antioxidant activity [4], as well as other biological properties [5–8]. Several human, animal and cell culture studies have shown that the phenolics of olive oil are bioavailable [7]. Although these previous studies clearly indicate cancer-protective activity and other health benefits of virgin olive oil, the mechanisms remain largely unknown. It was demonstrated that one of the olive oil

phenolics, tyrosol, protects to the Caco-2 cells from oxidized LDL-induced apoptotic cell death [9].

The aim of the present study was to investigate the potential inhibitory effects of OOPE and GA on ROS generation and apoptotic cell death in HeLa cells, which were treated or untreated with H_2O_2. Since these nonradical reactive oxygen species are considered potent mutagens and major mediators of DNA damage, determining the protective effect of olive oil against H_2O_2-induced apoptotic cell death could support the notion that olive oil has benefits for the health.

Materials and methods

Preparation of total phenolic extract from extra virgin olive oil

Extra virgin olive oil was purchased from a local market in Istanbul, Turkey. Extraction of total phenolic compounds from the extra virgin olive oil was carried out according to the methods reported by Nousis et al. [10]. The OOPE was dissolved in DMSO and stored in the dark at −20 °C.

Determination of total phenolic content

Total phenolic content was measured using the Folin–Ciocalteu's reagent, as described by Ragazzi and Veronese [11]. Gallic acid was used as a standard. Total phenolic content was expressed as mg gallic acid equivalent (GAE) per gram dry weight of OOPE, and GAE per kilogram of olive oil.

Cell culture

Human HeLa cervical carcinoma cells were grown in EMEM (Eagle's minimum essential medium with Earle's saline), supplemented with an antibiotic–antimycotic mixture [penicillin (100 U/mL), streptomycin (100 μg/mL), amphotericin B (0.25 μg/mL)], and 10% heat-inactivated fetal bovine serum. Cells were seeded at the concentration of 10^5 cells per milliliter and maintained at 37 °C in an atmosphere with 5% CO_2. OOPE or GA was added to the growth medium, after dissolving in DMSO at a final concentration not exceeding 0.5% (v/v), since DMSO is able to inhibit cell growth above this concentration (data not shown).

Cell cytotoxicity test

The MTT assay was used as has been previously described [12], in order to estimate cell viability. This method is based on the ability of viable cells to reduce 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) and to form a formazan product. The cells (10^5 cells/mL) were seeded into the well, containing 200 μL EMEM in a 96-well plate. Following this, the cells were treated with OOPE (0.0025-1 mg/mL) or GA (0.001–0.1 mg/mL) in the last 4, 24 and 48 h periods of a total incubation time of 72 h. At the end of the treatment period, the culture medium was removed and the cells were washed with phosphate-buffered saline (PBS) before 30 μL of MTT solution (5 mg/mL) was added to each well and the culture was incubated at 37 °C for 4 h. After the formation of formazan crystals, 200 μL of DMSO was added to the wells and the resulting optical density was measured using a microplate reader (μQuant, BioTek Instruments Inc., Winooski, VT, USA) at 570 and 690 nm (reference) wavelengths. The MTT reduction activity of cells was calculated as percentage of MTT reduction activity of cells in the sample-treated group versus an untreated control, using the following equation: MTT reduction activity (%) = $(OD_{sample}/OD_{control}) \times 100$.

OOPE and GA treatments

HeLa cells were incubated with noncytotoxic doses of test materials (95 μg/mL OOPE or 28 μg/mL GA) for the experimental groups or solely with EMEM for the untreated control in the last 4, 24 and 48 h periods of the total incubation time of 72 h.

H_2O_2 treatments

Two different doses of H_2O_2 were implemented with the OOPE- or GA-treated cells and the untreated control cells. ROS generation was enhanced by treating the cells with 200 μM H_2O_2. Harsh stress condition inducing apoptosis was created by 750 μM H_2O_2. Following the precise implementation of the test materials, the cells were washed with PBS, followed by an application of 200 μM H_2O_2 or 750 μM H_2O_2 for 1 h, for the induction of ROS generation or apoptosis, respectively. Hank's balanced salt solution was used for the dilution of H_2O_2 and as the blank in the assays, and the H_2O_2 concentration was checked by the absorbance measurement at 240 nm, as described by Aebi [13].

Measurement of intracellular ROS level

The intracellular ROS level was estimated by using a fluorescent probe, 2′,7′-dichlorofluorescein diacetate (DCFH-DA) [14]. At the end of the proper incubations and oxidative stress periods, the culture medium was immediately removed and the cells were washed with PBS followed by incubation for 15 min in 10 μM DCFH-DA (100 μL) at 37 °C in an atmosphere with 5% CO_2. The fluorescence of hydrolyzed 2,7-dichlorofluorescein (DCF)

bound to intracellular ROS was measured at 10 min intervals for 1 h in a microplate fluorometer with 485 excitation/530 nm emission wavelengths (FLx800™, Bio-Tek Instruments Inc., Winooski, VT, USA). The relative percentage of ROS production was calculated according to the following equation:

$$\mathrm{ROS}\ (\%) = (F_1/\ F_0)\ \mathrm{x}\ 100\%$$

$$\left(\begin{array}{l}\text{where } F_0:\ \text{fluorescence intensity of untreated control group;}\\ \qquad F_1:\ \text{fluorescence intensity of experimental group}\end{array}\right)$$

Measurement of caspase 9 activity

Caspase 9 activity was determined with the Caspase 9 Fluorometric Protease Assay Kit (Chemicon, USA and Canada). The assay is based on fluorometric detection of cleavage of substrate LEHD-AFC (Leu-Glu-His-Asp-7-Amino-4-trifluoromethyl coumarin). The substrate excites fluorescence at 400 nm. Upon cleavage, free AFC emits fluorescence that can be monitored at 505 nm. After 4 h incubation of HeLa cells with OOPE or GA, apoptosis was induced by 1 h incubation with 750 μM H_2O_2. Then, the cells were washed with PBS and cultured in fresh EMEM, and Caspase 9 activity was evaluated after 5, 15, 23 and 47 h following the 1 h incubation with H_2O_2. HeLa cells (1–2 × 10^6 cells) were disrupted in a lysis buffer by sonication, and the cell lysate was incubated with the indicated substrate in a reaction buffer for 1.5 h at 37 °C, as described in the manufacturer's instructions. Samples were read on a multi-well fluorescence plate reader (FLx800™, BioTek Instruments Inc., Winooski, VT, USA) at 400 nm excitation/505 nm emission wavelengths. The enzyme activity was normalized for per milligram protein in the cell lysate, determined with the BCA™ Protein Assay Kit (Pierce, Rockford, Illinois, USA) and relatively expressed as a percentage of control.

Statistical analysis

The data were expressed as mean ± standard error (S.E.) of three independent experiments conducted in triplicates. All analyses were carried out using GraphPad Prism (version 5.00 for Windows, GraphPad software Inc., San Diego, CA, http://www.graphPad.com.) software. Statistical analyses were performed using one-way ANOVA followed by the Dunnett multiple comparison test. The probability values of $p < 0.05$ were considered as significant.

Results and discussion

The present study examined the effects of olive oil phenolic extract and gallic acid on ROS generation and H_2O_2-induced apoptotic cell death in HeLa cells. A total of 3.695 ± 0.006 g OOPE was obtained per kg extra virgin olive oil. According to the results obtained by the Folin–Ciocalteu method, the total phenolic content of OOPE and extra virgin olive oil was 5.46 ± 0.1 mg GAE/g dry OOPE and 19.76 ± 0.5 mg GAE/kg olive oil, respectively. The phenolic compounds are secondary plant metabolites biosynthesized through the shikimic acid pathway [15]. These compounds have antioxidant properties because of their ability to scavenge free radicals and active oxygen species such as singlet oxygen, free radicals and hydroxyl radicals [16, 17]. Olives and olive oils are a good source of several phenolic compounds, such as hydroxytyrosol, tyrosol, gallic acid, caffeic acid and oleuropein [5, 18].

The cytotoxic effect of OOPE and GA on the HeLa cells was investigated with an MTT assay, based on the reduction in MTT to a colored formazan product by mitochondrial dehydrogenase, which is active only in living cells. OOPE or GA decreased the viability of HeLa cells in dose- and time-dependent manners for all treatments (Fig. 1). It is known that relatively low concentrations of OOPE protect DNA from oxidative damage in Jurkat cells, HL60 cells and human peripheral blood mononuclear cells exposed to hydrogen peroxide [10, 19]. However, high doses are able to induce apoptosis in HL60 cells [20]. Similarly, the olive oil phenolic extract exhibited genotoxic effects on the Jurkat cells at 100 μg/mL or higher concentrations [10]. In addition, high concentrations of GA are known to induce apoptosis in some cells [21, 22]. Thus, noncytotoxic doses (95 μg/mL for OOPE and 28 μg/mL for GA) were used in this study in order to avoid the cytotoxic effects of OOPE and GA. The intracellular ROS production was estimated by using a fluorescent probe DCFH-DA for the prediction of the oxidative stress grade in the cells. The DCFH-DA readily diffuses through the cell membrane and is enzymatically hydrolyzed by intracellular esterases to form nonfluorescent 2′,7′-dichlorofluorescein (reduced form) (DCFH), which is then rapidly oxidized to form highly fluorescent 2′,7′-DCF in the presence of ROS. The results of this study showed that the 1 h treatment of 200 μM H_2O_2 induced ROS generation in HeLa cells, and OOPE and GA treatments significantly decreased the steady-state generation of ROS, thus preventing or delaying conditions that favor oxidative stress in the cells (Table 1). The incubation of the cells with 200 μM H_2O_2 caused statistically significant (paired *t* test; one-tailed *p* value) increased levels of ROS in HeLa cells. Compared to untreated cells, intracellular ROS production was higher by 19.2% in H_2O_2-treated cells. In contrast, no increased levels of ROS were observed in the cells pretreated by OOPE and GA for 4, 24 and 48 h before oxidative stress induction with H_2O_2. Moreover, the ROS level of HeLa cells, which were pretreated with OOPE and

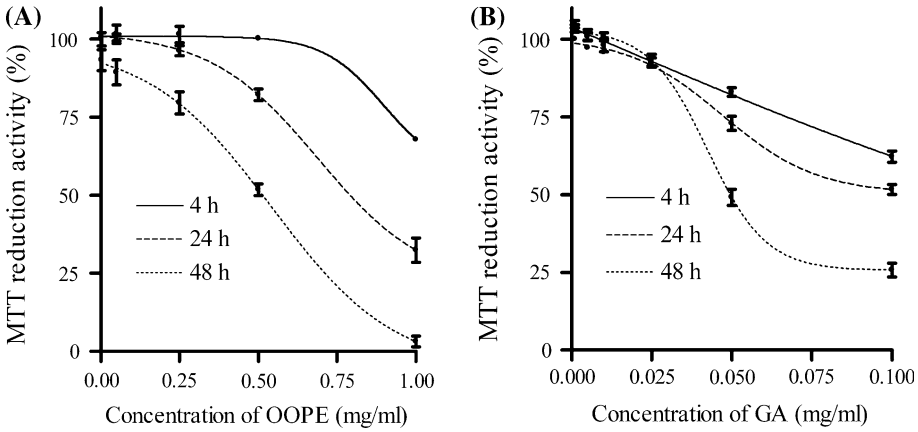

Fig. 1 Effect of pretreatment times and increasing concentrations of OOPE (olive oil phenolic extract) (**a**) and GA (gallic acid) (**b**) on MTT reduction activity of HeLa cells

GA for 4 h and 24 h, was lower than the basal ROS level. The present study also showed that the ROS inhibitory effect of OOPE was gradually decreased as the incubation time was prolonged, and this probably indicates its metabolic inactivation. Indeed, it has been proposed that glutathionylation and O-methylation of catecholic compounds take place intracellularly [10, 23]. The most interesting finding was that the best results were recorded for the short period (4 h) treatments under harsh stress conditions. Thus, it seemed that HeLa cells were protected against ROS attack through a direct antioxidant effect of OOPE and GA. These results were consistent with previous reports, indicating olive oil has a protective effect against oxidant agents- and radiation-induced oxidative damage in different cell lines [19, 24–28].

Oxidative stress has been defined as a disturbance in the prooxidant/antioxidant balance, resulting in potential cellular damage [29]. It has been implicated in several biological and pathological processes like aging, inflammation, carcinogenesis, ischemia–reperfusion, and in some diseases including AIDS, Parkinson's, Huntington's, familial amyotrophic lateral sclerosis and cataract formation in the eye. Apoptosis has been associated with these diseases and oxidative stress can modulate the apoptotic pathway [30].

Apoptosis is an inherent cellular response for effective cellular disposal against development and environmental insults. It can be induced by diverse stimuli, and common signaling mediators, including ROS, which induce DNA damage [31]. It has been shown that high concentration of H_2O_2 induces necrosis, whereas a low concentration induces apoptosis [32]. In another study, it was reported that apoptosis was triggered by H_2O_2 via the mitochondrial pathway involving upregulation of p73 and down regulation of anti-apoptotic Bcl-XL, the release of cytochrome c from mitochondria and activation of caspases 9 and 3 in HeLa cells [33]. Activation of caspases by cytochrome c is a key event during apoptosis caused by various toxic agents [34]. We measured the alteration of caspase 9 activity in HeLa cells to determine whether the apoptosis was induced in our assay system. The caspase 9 activity increased in a time-dependent manner, and maximum activation occurred at the 23 h of incubation after the treatment with H_2O_2. The results are given in Fig. 2. In the H_2O_2 untreated culture, incubation with OOPE decreased the caspase 9 activity by approximately 11% compared to untreated control cells, whereas GA did not cause any alteration in caspase 9 activity (Fig. 3). A significant (~117%) increment in enzyme activity was observed following the apoptosis induction. The depressing effect of OOPE was very distinctive (92%) under harsh stress conditions (Fig. 3). GA also significantly suppressed (67.8%) the enzyme activity, but not as well as OOPE. These results clearly indicated

Table 1 The effect of OOPE (olive oil phenolic extract) and GA (gallic acid) pretreatments on intracellular ROS generation

	p.t. (h)	Relative amount of intracellular ROS (%)	
		No stress induction (H_2O_2 = 0 μM)	Stress induction (H_2O_2 = 200 μM)
Untreated control		100.00 ± 0.29	119.20 ± 1.72**
OOE (95 μg/mL)	4	90.05 ± 1.69**	89.63 ± 2.77**●
	24	92.30 ± 2.58*	94.41 ± 3.68●
	48	103.20 ± 1.19	106.50 ± 1.45●
GA (28 μg/mL)	4	91.34 ± 1.69**	92.38 ± 2.94**●
	24	93.01 ± 2.69*	95.13 ± 3.18●
	48	94.80 ± 2.96	99.97 ± 2.41●

p.t. pretreatment time as hours; * $p < 0.05$; ** $p < 0.01$ in comparison with untreated control; ● $p < 0.01$ in comparison with 200 μM H_2O_2

that OOPE and GA were able to inhibit the caspase-dependent apoptosis pathway in HeLa cells. Fabiani et al. [19] suggest that high concentrations of OOPE induced apoptotic cell death, while low concentrations of the phenolic constituents, whether or not in a complex mixture, prevent H_2O_2-induced DNA damage [10, 19]. Fabiani and Morozzi [35] have recently concluded that the DNA protection effects of olive oil mainly result from the antioxidant activity of phenolics. Similarly, Sohi et al. [18] showed that the anti-apoptotic effect provided by gallic acid against oxidative stress in human peripheral blood lymphocytes was due to its direct action in the scavenging of free radicals.

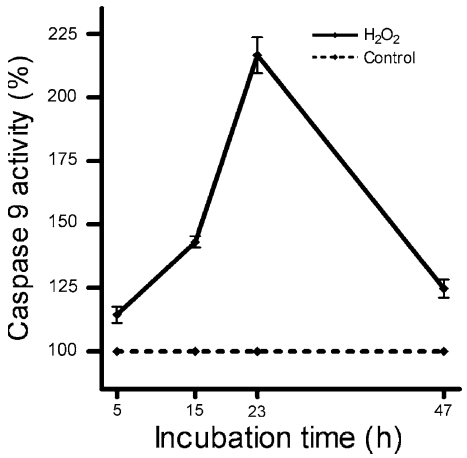

Fig. 2 Time-dependent variation of caspase 9 activity with hydrogen peroxide implementation in HeLa cells

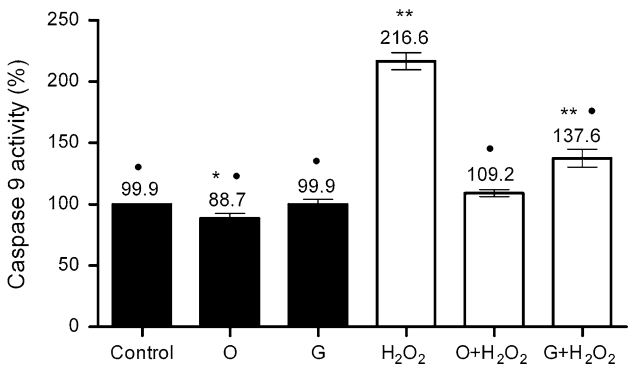

Fig. 3 Caspase 9 activity in HeLa cells 23 h after 1 h treatment/untreatment with 750 μM H_2O_2 and/or 4 h pretreatment/unpretreatment with OOPE (olive oil phenolic extract)/GA (gallic acid). *Control*: Untreated cells; *O*: Cells pretreated 4 h with 95 μg OOPE/mL; *G*: Cells 4 h pretreated with 28 μg GA/mL; H_2O_2: Cells 1 h treated with 750 μM H_2O_2; $O + H_2O_2$: Cells pretreated with 95 μg OOPE/mL followed by 1 h treated with 750 μM H_2O_2; $G + H_2O_2$: Cells pretreated with 28 μg GA/mL followed by 1 h treated with 750 μM H_2O_2. $^*p < 0.01$, $^{**}p < 0.001$; mean values were significantly different from the untreated control. $^\bullet p < 0.001$; mean values were significantly different from 750 μM H_2O_2

In conclusion, using the in vitro assay system described in this study, OOPE and GA were proved to be potent ROS inhibitors as well as apoptosis deterrents in HeLa cells, especially under harsh stress conditions. These findings demonstrate that olive oil phenolic extract acts as a protective and antioxidant agent against the deleterious effect of oxidative stress. The concentration of OOPE used in this study might be present in the intestine after the consumption of olive oil; therefore, the findings could provide information about the protective effects of olive oil against ROS in the intestine. However, the concentration used in the study could be higher than the one within the vascular system or tissues. It would be of great benefit to evaluate the utility for health of olive oil phenolic extract as a whole mixture of many phenolic compounds rather than as a single nutritional supplement. These findings underscore the importance of continuing research into the synergistic effect of olive oil phenolics and comparing the phenolic contents of various olive oils that link the diet with a healthy life in humans.

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Conflict of interest The authors have declared no conflict of interest.

References

1. Halliwell B (1994) Free radicals and antioxidants: a personal view. Nutr Rev 52:253–265
2. Ames BN, Shigenaga MK, Hagen TM (1993) Oxidants, antioxidants and the degenerative disease of aging. PNAS USA 90:7915–7922
3. Bohr VA (2002) Repair of oxidative DNA damage in nuclear and mitochondrial DNA, and some changes with aging in mammalian cells. Free Radical Bio Med 32(9):804–812
4. Carrasco-Pancorbo A, Cerretani L, Bendini A et al (2005) Evaluation of the antioxidant capacity of individual phenolic compounds in virgin olive oil. J Agric Food Chem 53(23):8918–8925
5. Visioli F, Galli C (2002) Biological properties of olive oil phytochemicals. Crit Rev Food Sci Nutr 42:209–221
6. Roos B, Zhang X, Gutierrez GR (2011) Anti-platelet effects of olive oil extract: in vitro functional and proteomic studies. Eur J Nutr 50(7):553–562
7. Vissers MN, Zock PL, Katan MB (2004) Bioavailability and antioxidant effects of olive oil phenols in humans: a review. Eur J Clin Nutr 58:955–965
8. Covas MI, Nyyssönen K, Poulsen HE et al (2006) The effect of polyphenols in olive oil on heart disease risk factors. Ann Intern Med 145:333–341
9. Giovannini C, Straface E, Modesti D, Coni E, Cantafora A, De Vincenzi M, Malorni W, Masella R (1999) Tyrosol, the major olive oil Biophenol, protects against oxidized-LDL-induced injury in Caco-2 cells. J Nutr 129:1269–1277
10. Nousis L, Doulias PT, Aligiannis N, Bazios D, Agalias A, Galaris D, Mitakou S (2005) DNA protecting and genotoxic effects of

olive oil related components in cells exposed to hydrogen peroxide. Free Radical Res 39(7):787–795
11. Ragazzi E, Veronese G (1973) Quantitative analysis of phenolic compounds after thin-layer chromatographic separation. J Chromatogr 77:369–375
12. Mosmann T (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxic assays. J Immunol Methods 65(1–2):55–63
13. Aebi H (1974) Catalase. In: Bergmeyer HU (ed) Methods of enzymatic analysis. Verlag Chemie Weinheim, Germany, pp 673–684
14. Negre-Salvayre A, Auge N, Duval C, Robbesyn F, Thiers JC, Nazzal D, Benoist H, Salvayre R (2002) Detection of intracellular reactive oxygen species in cultured cells using fluorescent probes. Methods Enzymol 352:62–71
15. Muzzalupo I, Stefanizzi F, Perri E, Chiappetta AA (2011) Transcript levels of CHL P gene, antioxidants and chlorophylls contents in olive (*Olea europaea* L.) pericarps: a comparative study on eleven olive cultivars harvested in two ripening stages. Plant Foods Hum Nutr 66(1):1–10
16. Cheung SC, Szeto YT, Benzie IF (2007) Antioxidant protection of edible oils. Plant Foods Hum Nutr 62(1):39–42
17. Saito K, Kohno M, Yoshizaki F, Niwano Y (2008) Extensive screening for edible herbal extracts with potent scavenging activity against superoxide anions. Plant Foods Hum Nutr 63(2):65–70
18. Sohi KK, Mittal N, Hundal MK, Khanduja KL (2003) Gallic acid, an antioxidant, exhibits antiapoptotic potential in normal human lymphocytes: a Bcl-2 independent mechanism. J Nutr Sci Vitaminol (Tokyo) 49(4):221–227
19. Fabiani R, Rosignoli P, De Bartolomeo A, Fuccelli R, Servili M, Montedoro GF, Morozzi G (2008) Oxidative DNA damage is prevented by extracts of olive oil, hydroxytyrosol, and other olive phenolic compounds in human blood mononuclear cells and HL60 cells. J Nutr 138:1411–1416
20. Fabiani R, De Bartolomeo A, Rosignoli P et al (2006) Virgin olive oil phenols inhibit proliferation of human promyelocytic leukemia cells (HL60) by inducing apoptosis and differentiation. J Nutr 136:614–619
21. You BR, Park WH (2010) Gallic acid-induced lung cancer cell death is related to glutathione depletion as well as reactive oxygen species increase. Toxicol In Vitro 24(5):1356–1362
22. Hsu CL, Lo WH, Yen GC (2007) Gallic acid induces apoptosis in 3T3–L1 pre-adipocytes via a fas- and mitochondrial-mediated pathway. J Agric Food Chem 55:7359–7365
23. Spencer PE, Kunhle CGG, Williams JR, Rice-Evans C (2003) Intracellular metabolism and bioactivity of quercetin and its in vivo metabolites. Biochem J 372:173–181
24. D'Angelo S, Ingrosso D, Migliardi V et al (2005) Hydroxytyrosol, a natural antioxidant from olive oil, prevents protein damage induced by long-wave ultraviolet radiation in melanoma cells. Free Radical Bio Med 38:908–919
25. Di Benedetto R, Vari R, Scazzocchio B et al (2007) Tyrosol, the major extra virgin olive oil compound, restored intracellular antioxidant defences in spite of its weak antioxidative effectiveness. Nutr Metabol Cardiovasc Dis 17:535–545
26. Goya L, Mateos R, Bravo L (2007) Effect of the olive oil phenol hydroxytyrosol on human hepatoma HepG2 cells. Eur J Nutr 46:70–78
27. Zhang X, Cao J, Jiang L, Zhong L (2009) Suppressive effects of hydroxytyrosol on oxidative stress and nuclear factor-κB activation in THP-1 cells. Biol Pharm Bull 32(4):578–582
28. Guo W, An Y, Jiang L, Geng C, Zhong L (2010) The protective effects of hyrodxytyrosol against UVB-induced DNA damage in HaCaT cells. Phytother Res 24:352–359
29. Sies H (1985) Oxidative stress: introductory remarks. In: Sies H (ed) Oxidative stress. Academic Pres, London, pp 1–8
30. Chandra J, Samali A, Orrenius S (2000) Triggering and modulation of apoptosis by oxidatıve stress. Free Radical Bio Med 29(3/4):323–333
31. Rodriguez H, Holmquist GP, D'Agostino R, Keller J, Akman SA (1997) Metal ion-dependent hydrogen peroxide-induced DNA damage is more sequence specific than metal specific. Cancer Res 57:2394–2403
32. Troyano A, Sancho P, Fernandez C, De Blas E, Bernardiand P, Aller P (2003) The selection between apoptosis an d necrosis is differentially regulated in hydrogen peroxide-treated and glutathione-depleted human promonocytic cells. Cell Death Differ 10:889–898
33. Singh M, Sharma H, Singh N (2007) Hydrogen peroxide induces apoptosis in HeLa cells through mitochondrial pathway. Mitochondrion 7:367–373
34. Green DR, Reed C (1998) Mitochondria and apoptosis. Science 281:1309–1312
35. Fabiani R, Morozzi G (2010) Anticarcinogenic properties of olive oil phenols: effects on proliferation, apoptosis and differentiation. In: Preedy VR, Watson RR (eds) Olives and olive oil in health and disease prevention. Academic Press, London, pp 981–988