Original Article

Antioxidant Properties and Protective Effect of Turkish Propolis on t-BHP-Induced Oxidative Stress in Foreskin Fibroblast Cells

Sema Misir[1,2*], Yuksel Aliyazicioglu[3], Selim Demir[4], Ibrahim Turan[5,6], Serap Ozer Yaman[2], Orhan Deger[3]

[1]Department of Biochemistry, Faculty of Pharmacy, Cumhuriyet University, Sivas, TURKIYE.
[2]Department of Medical Biochemistry, Institute of Health Sciences, Karadeniz Technical University, Trabzon, TURKIYE.
[3]Department of Medical Biochemistry, Faculty of Medicine, Karadeniz Technical University, Trabzon, TURKIYE.
[4]Department of Nutrition and Dietetics, Faculty of Health Sciences, Karadeniz Technical University, Trabzon, TURKIYE.
[5]Department of Genetic and Bioengineering, Faculty of Engineering and Natural Sciences, Gumushane University, Gumushane, TURKIYE.
[6]Medicinal Plants, Traditional Medicine Practice and Research Center, Gumushane University, Gumushane, TURKIYE.

ABSTRACT

Objective: Propolis is an important bee product, rich in polyphenolic compounds. It has antitumoral, antioxidant, antimutagenic, and other useful activities. Biological activities of propolis are generally attributed to its substance of polyphenolic compounds. The aim of this study was to investigate the *in vitro* antioxidant properties and reduction amount of intracellular reactive oxygen species (ROS) in human normal foreskin fibroblast cells by Turkish propolis ethanolic extract (EEP). **Method:** Total phenolic and flavonoid contents, reducing power, radical scavenging capacity, and iron chelating activity of EEP were determined using spectrophotometric methods. Intracellular ROS levels were determined by spectrofluorometric analysis with CM-H_2DCFDA. **Results**: Our results indicated that antioxidant activity was correlated with the higher total phenolic and flavonoid contents of EEP. The generated ROS levels were significantly decreased by EEP compared to only t-BHP group. **Conclusion:** Ethanolic propolis extract may be evaluated as a potency antioxidant resource and/or a novel natural agent in food and drug industries.

Key words: Antioxidant activity, CM-H_2DCFDA, Fibroblast cells, Polyphenols, Propolis.

INTRODUCTION

Human beings have recognized and have benefited from the therapeutic aspect of natural products thousands years ago. In recent years, the emergence of the side effects of the drugs has led people to the consumption of natural products known as medicine. One of the most widely used products is propolis, which is a bee product.[1] Propolis is quite rich in polyphenolic compounds (caffeic acid, ferulic acid, etc.), flavonoids (quercetin, rutin, pinocembrin, naringenin, crysin, etc.) and contains many vitamins, minerals, and trace elements.[2,3,4] Propolis has been used in traditional medicine for many years for a variety of purposes.[5] Bee products are used to prevent the progression of the disorders, to reduce pain and to treat the disease.[1,5] Valuable information have been revealed about antioxidant, antibacterial, antiviral, anti-inflammatory, anticancer, antimutagenic, radioprotective, and other beneficial biological effects and chemical structure of propolis.[6,7,8,9,10] In recent years, propolis has become quite popular in pharmaceutical, cosmetic, and food industries because of above-mentioned properties that it possesses.[6,11]

Flavonoids are natural polyphenolic compounds that can be found in fruits, vegetables,

Submission Date: 08-03-2017;
Revision Date: 31-03-2017;
Accepted Date: 28-07-2017

DOI: 10.5530/ijper.52.1.11

Correspondence:
Dr. Sema Misir,
Department of Biochemistry, Faculty of Pharmacy, Cumhuriyet University, 58140, Sivas, TURKIYE.
Phone no: +90 346 2191010 ext. 3913
E-mail: semay-61@hotmail.com

www.ijper.org

and beverages, such as tea, wine, and fruit juice.[12] Since these compounds are secondary plant metabolites, they cannot be synthesized by humans and so they are forming a significant part of human diet.[13] Flavonoids display their biological activities by bonding heavy metal ions and biological polymers, by catalyzing electron transport and scavenging free radicals.[13,14] Recent studies have showed that flavonoids protect DNA and other components of the cell from oxidative damage.[10,15,16]

Free radicals are defined as atoms or groups of atoms that includes an unpaired electrons. Reactive oxygen species (ROS) are described as chemically reactive molecules that include one or more oxygen atoms, which contain free radicals and non-radical reactive compounds that can oxide biological molecules. Free radicals give rise to many damages in cells and tissues.[17] Numerous studies have evaluated the antioxidant activity and beneficial biological effects of propolis.[2,8,9,13,16] Propolis shows antioxidant properties because of its free radical scavenging capacity, which aroused the need of conducting further researches about them in many areas.[16]

In our previous study[16] we determined that ethanolic extract of Turkish propolis (EEP) (200 μg/mL) reduces the damage induced by t-BHP in fibroblast cells after 4 h recovery period. EEP reduce that the amount of intracellular ROS has not been shown any study revealed in fibroblast cells. The purpose of this study was to investigate *in vitro* antioxidant properties and also for the first time the protecting effect of Turkish propolis on t-BHP-induced oxidative stress in fibroblast cells was evaluated.

MATERIALS AND METHODS

Chemicals

All chemicals used for antioxidant activity analysis were analytical grade and purchased from Sigma (St. Louis, MO, USA). All chemicals used for cell culture studies were obtained from Lonza (Verviers, Belgium) and Biological Industries (Kibbutz Beit Haemek, Israel). 5-(and-6)-chloromethyl-2',7'-dichlorodihydrofluoresceindiacetate acetyl ester (CM-H_2DCFDA) was purchased from Invitrogen (Paisley, UK).

Preparation of EEP

First, 0.5 g of propolis powder, which has been harvested from Trabzon and its surrounding, was solved into 20 mL pure ethanol, after vortexing, they were held in incubation, at 60°C, 150 rpm in a mechanical shaker for 24 h. EEP was filtered by a filter of 0.2 μm and stored at -20°C until used.

Total Polyphenolic Content (TPC)

Content of total polyphenols were measured with Folin-Ciocalteu method adapted to microscale, as described by Slinkard and Singleton.[18] The method of based on phosphotungstic acid reduction reaction in the basic solution. 12.5 μL EEP, 62.5 μL Folin-Ciocalteu reagent, and 125 μL 20% aqueous sodium carbonate were mixed. The final mixtures were incubated for 30 min at 25°C. The results were expressed as mg gallic acid equivalents (GAE)/g sample.

Total Flavonoid Content (TFC)

Content of total flavonoids were determined by colorimetric method in a 96-well microplate, as described by Chang *et al.*[19] 20 μL EEP, 172 μL 80% ethanol, 4 μL 10% aqueous $Al(NO_3)_3$, 4 μL 1 M KCH_3COO were mixed and incubated for 40 min. The values were expressed as mg quercetin equivalents (QE)/g sample.

Reducing Power

The reducing antioxidant power were evaluated by spectrophotometric method.[20] Briefly, 40 μL EEP, 100 μL 0.2 M phosphate buffer, 100 μL 1% $K_3Fe(CN)_6$ were mixed and incubated for 20 min at 50°C. Then, 100 μL 10% TCA was added to the tubes. Tubes were centrifuged at 3000 g for 10 min. 100 μL supernatants, 100 μL distilled water, 20 μL 0.1% $FeCl_3$ were mixed and incubated for 5 min. The results were expressed as mg trolox equivalents (TE)/g sample.

Determination of Radical Scavenging Activity

For the scavenging activity of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical by EEP, the method developed by Yu *et al.* was used.[21] When treated by an antioxidant substance or substances, the intensity of the purple color originated from DPPH is reduced and absorbance values decrease. In this study, 0.1 mM ethanolic DPPH solution was used. EEP were prepared in different concentrations. Briefly, equal volumes (750 μL) of DPPH and sample solutions were mixed and incubated for 50 min. To compare the outcomes, a natural antioxidant ascorbic acid was used. Radical scavenging activity (RSA) of the samples and the standard were given as their percentage (%) to negative control (DPPH alone) and they were calculated using the Equation 1.

$$RSA\ (\%) = [(A_{control} - A_{sample})/A_{control}] \times 100$$

Determination of Metal Chelating Activity

For ferrous ions (Fe^{2+}) chelating activity of EEP, a modified version of the method developed by Dinis *et al.* was used. Metal chelating activity was assessed by comparing its ability to bind Fe^{2+} ions in the solution with ferrozine.[22]

Briefly, 50 µL EEP, 50 µL 0.2 mM $FeCl_2$ solution, 200 µL 5 mM ferrozine solution, and 1.7 mL distilled water were mixed and incubated for 10 min. EDTA, which is a good metal chelator, was selected as the standard. The pure solvent of the sample was used as the negative control. Iron binding capacity of the sample and the standards were given as their percentage (%) to negative control and control and they were calculated using the Equation 2.

$$\text{Inhibition (\%)} = [(A_{control}-A_{sample})/A_{control}] \times 100$$

Cell Culture

Human foreskin fibroblast cells (CRL-2522) were obtained from America Type Culture Collection (Manassas, VA). Cells were cultured in DMEM containing 10% FBS, 1% antibiotic solution in an incubator at 37°C.

Spectrofluorometric Analysis of Intracellular Oxidative Stress with CM-H_2DCFDA

5-(and-6)-chloromethyl-2',7'dichlorodihydrofluorescein diacetate, acetyl ester that was oxidized radicals was used to measure ROS generation occurred in our cell samples. 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) is deacetylated by esterase and it is transformed into a dichlorofluorescein (2',7'-DCFH) product that doesn't have fluorescent property. DCFH is a probe that is transformed to DCF (dichlorofluorescein) by ROS in the media giving a strong fluorometric emission. Fluorescence intensity can be identified spectrofluorometrically.[23] 5000 cells were plated in each well of 96 wells cell culture plate in 200 µL media. After 24 h, the media of the plate were removed and cells were treated with 200 µg/mL EEP, 15 mM deferoxamine mesylate (DFO), and 200 µM ascorbic acid for 1 h at 37°C in the new media. Next, the media of the plate was removed and new media was added to the plate. 300 µM t-BHP was added and incubated at 37°C for 1 h. The media of the plate was removed and new media was added to the wells and incubated at 37°C for 4 h[16], CM-H_2DCFDA dye was added to the plate, in a way to adjust the final concentration of the media to 10 µM, incubated at 37°C for 10 min. Fluorescence intensity was recorded at λ_{em}: 527 nm, λ_{ex}: 492 using a microplate reader fluorometer (Molecular Devices, Sunnyvale, CA). The results were given as relative fluorescence intensity according to negative control sample.

Statistical Analysis

All experiments were studied at triplicate. Descriptive statistical analysis was performed for all the studied variables. Data were given as mean±SD for normally distributed variables. ANOVA was used to compare parameters among groups and Tukey's test was performed for post-hoc comparisons. Statistical significance was set at $p<0.05$.

RESULTS

The value for the results of TPC, TFC, and reducing power of EEP are given in Table 1. Radical scavenging activities of EEP were studied through DPPH determination. % inhibition graphic of EEP which are radical scavenging activity are shown in Figure 1. The highest RSA of EEP was observed at the concentration of 125 µg/mL. Concentration % inhibition graphic showing metal chelating activity of EEP and EDTA were presented in Figure 2. The highest metal chelating activity of EEP was observed at the concentration of 25 mg/mL. Relative density percentage of ROS production is given in Table 2. At the end of 4 h treatment performed on fibroblast cells, which were damaged with 300 µM t-BHP, it was observed that 200 µg/mL EEP, 15 mM DFO, and 200 µM ascorbic acid significantly reduced the amount of generated ROS compared to the positive control (p values; 0.0001, 0.0001, and 0.001, respectively).

DISCUSSION

Many studies discuss the biological properties and the composition of propolis have raised the interest of the researchers towards this bee product.[1,24,25]Also in our study, we used total propolis extract instead of isolating one of the flavonoids included in the propolis. Biological activity of propolis mainly ascribed to its content of flavonoids and various phenolic compounds.[26,27] There are many methods for extraction of phenolic components from plant samples. Maceration is well known that the methods is used for extraction of the bioactive components. In this method different solvents (ethanol, methanol, DMSO, etc.) are used to extract the components

Table 1: Antioxidant properties of EEP.

	Total Phenolic Content mg GAE/g sample	Total Flavonoid Content mg QE/g sample	Reducing Power mg TE/g sample
Ethanolic Extract	114.7±0.02	36.02±0.08	246.8±0.01

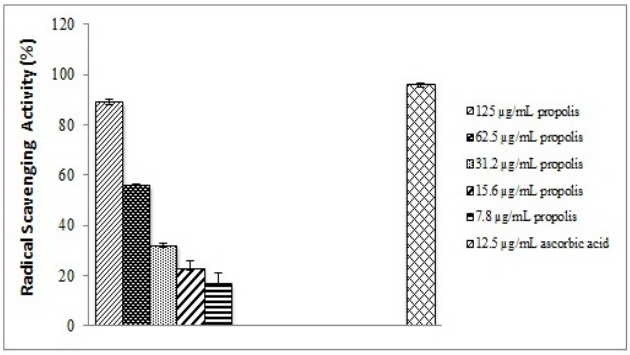


Figure 1: Concentration % inhibition graphic for the scavenging of DPPH free radical.

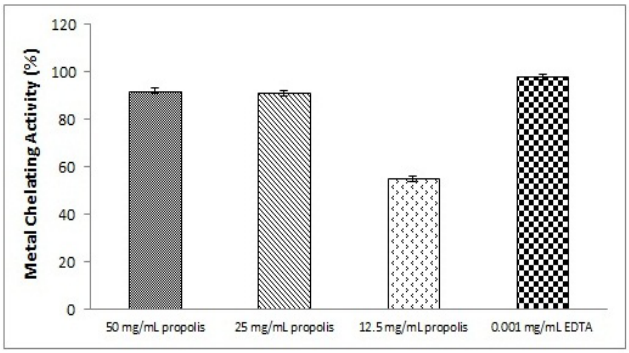


Figure 2: Concentration % inhibition graphic showing metal chelating activity.

Table 2: Intracellular ROS levels in human fibroblast cells measured by spectrofluorometric method.

Test Compounds	Relative density percentage (%)
Negative control	99±1.4[a]
200 µg/mL Propolis + 300 µM t-BHP	99.6±2.4[a]
15 mM DFO + 300 µM t-BHP	94.3±0.01[a]
200 µM ascorbic acid + 300 µM t-BHP	117.1±6.0[b]
Positive control	130±1.6

Represent significant results ([a]p=0.0001, [b]p=0.001) compared to positive control (300 µM t-BHP alone).
Values are mean±SD (n=3)

from plant samples directly without compromising the structure. It is known that ethanolic propolis extracts are one of the richest sources of phenolic and flavonoid compounds.[24] In this study, the ethanolic extract of Turkish propolis was prepared by the maceration methods.[28] Various *in vitro* assay can be used to determine antioxidant activity of natural product extracts.[28] In our study the values of TPC and TFC of EEP were found 114.7 mg GAE/g powder and 36.02 mg QE/g powder should be changed with GAE/g powder and 36.02 mg QE/g powder, respectively. Mihai *et al.*[29] investigated TPC values of various propolis samples from Transylvania and these values were changed between 24.46 and 62.39 g standards mixture/100 g of propolis. In another study, Can *et al.* were analyzed of propolis from 15 different locations in Azerbaijan. TPC values of these samples was between 10.94 and 79.23 mg GAE/g propolis.[30] TPC and TFC values of propolis extracts have been reported that as 174.7 mg GAE/g dry sample, 45.1 mg QE/g dry sample from China,[31] between 150-197 mg GAE/g dry sample, 35.64-62.04 mg QE/g dry sample from Poland,[32] and 31.2-299 mg GAE/g dry sample, 2.5-176 mg QE/g dry sample from other parts of the world, respectively.[2] Reducing antioxidant potential was used to determine antioxidant activity of the propolis.[2] It was found that reducing power values of EEP was 246.8 mg trolox equivalents. FRAP values of propolis have been reported at between 170-438 µM trolox/g dry sample from 15 different locations in Azerbaijan,[30] between 0.74-2.54 mmol Fe_2SO_4/g propolis from Transylvania.[29]

Free radical scavenging activity of propolis was tested through DPPH, which is a stable radical. It is known that the solvents used for extraction are also effective in the radical scavenging activity. It was observed that EEP were quite effective, even in low concentrations, such as 125 µg/mL. Geckil *et al.*[33] showed that radical scavenging activity of ethanolic propolis extracts are over 90% for the concentration of 200 µg/mL and over. Siripatrawan *et al.*[34] evaluated that free radical scavenging activity of ethanolic propolis extracts are 94.2% for the concentration of 30 mg/mL. It's clear that results of TPC, TFC, radical scavenging activity, and reducing power analysis were generally similar to those of other studies in literature.

It was found that metal chelating activity of EEP was around 90% for the concentration of 25 mg/mL and more. Metal chelating activity of different propolis extracts have been reported as 2 mg/mL has showed around 50% inhibition from Brazilian[35], 70% inhibition for the concentration of 2 mg/mL from Malatya, Turkey[33] and 2 mg/mL has showed around 55.9% inhibition from Slovenian.[36] Study of Miguel *et al.* reported that chelating Fe^{2+} ions activity of methanolic propolis extract has

between 4.33-29.68% for the concentration of 100 µg/mL from Algarve, South Portugal.[37] Our metal chelating activity results were different from the previous reports. This situation may arise from harvested region, type of solvents, and extraction methods.[28]

Various chemicals, such as hydrogen peroxide (H_2O_2), t-BHP, methyl methanesulfonate, and ferrous sulfate are used in cells to generate ROS *in vitro*.[16,26,38] t-BHP was selected as the chemical to be used for generating ROS. To measure the production of ROS in cells, CM-H_2DCFDA was used. The ROS levels were significantly reduced with EEP pre-treatment compared to positive control (p=0.0001). DFO and ascorbic acid were used in ROS studies as controls due to their scavenging and metal chelating activities.[39,40] ROS amounts were significantly reduced with both DFO and ascorbic acid compared to positive control. In the literature, there are many studies showing that both extract of natural compounds and single polyphenolic compounds decrease intracellular ROS amount and protect biomolecules from oxidative stress.[38,41] Sharon *et al.* reported that quercetin and rutin prevent t-BHP induced oxidative damage in Caco-2 cells.[39] Kang *et al.* demonstrated that caffeic acid prevents H_2O_2-induced damage in WI-38 cells due to decreasing intracellular ROS and increasing catalase activity with its radical scavenging activity.[23] Yen *et al.* reported that ascorbic and gallic acid prevents H_2O_2-induced oxidative damage in lymphocytes through decreasing DNA damage with their radical scavenging activity.[40] Wang *et al.* reported that caffeic acid phenethyl ester (CAPE) and its derivatives prevent endothelial cells against menadione-induced oxidative damage.[43] In another study, Song *et al.* demonstrated that CAPE prevent H_2O_2-induced oxidative stress in human middle ear epithelial cells due to decreasing intracellular ROS.[42] Dhanya *et al.* found that quercetin prevents t-BHP induced oxidative stress in L6 myoblasts[44], while Lee *et al.* demonstrated that CAPE protects human liver (HepG2) cells against t-BHP induced oxidative stress through decreasing amount of intracellular ROS.[45]

Mentioned above in literature showed that reduction intracellular ROS in different cell lines both by each extract components of propolis and total propolis extract were reported and similar results was found for EEP effect in this particular study. The reduction in intracellular ROS amount by propolis extracts may be because of the flavonoids and phenolics that it contains, which is unique to the region. Polyphenols show their antimutagenic and anticarcinogenic effects not only that by preventing the spread of free radicals, but also by chelating the transition metals in the environment and inhibiting radical generating reactions, therefore by preventing radical generation.[46]

CONCLUSION

Consequently, our results demonstrated that EEP reduced the amount of intracellular ROS due to its phenolic content, radical scavenging, and metal chelating activity. Thus, EEP might be evaluated a novel potential antioxidant source and a therapeutic agent for drug industries.

ACKNOWLEDGEMENT

This study was supported by the Karadeniz Technical University Scientific Research Foundation (Trabzon, Turkey).

CONFLICTS OF INTEREST

The authors declare that there are no conflict of interest.

ABBREVİATİONS USED

ROS: Reactive Oxygen Species; **EEP:** Turkish Propolis Ethanolic Extract; **DPPH:** 2, 2-diphenyl-1-picrylhydrazyl; **CM-H_2DCFDA:** 5-(and-6)-chloromethyl-2',7'-dichlorodihydrofluorescein diacetate, acetyl ester; **TPC:** Total Phenolic Content; **TFC:** Total Flavonoid Content; **FRAP:** Ferric Reducing Antioxidant Potential.

REFERENCES

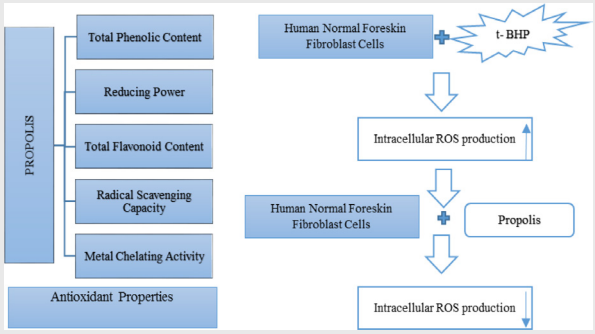
1. Sforcin JM, Bankova V. Propolis is there a potential for the development of new drugs? J Ethnopharmacol. 2011; 133(2): 253-60.
2. Turan I, Demir S, Misir S, Kilinc K, Mentese A, Aliyazicioglu Y, *et al.* Cytotoxic effect of Turkish propolis on liver, colon, breast, cervix and prostate cancer cell lines. Trop J Pharm Res. 2015; 14(5): 777-782.
3. Bankova V, Castro SL, Marcucci MC. Propolis: recent advances in chemistry and plant origin. Apidologie 2000; 31(1): 3-15.
4. Kurek-Górecka A, Rzepecka-Stojko A, Górecki M, Stojko J, Sosada M, Świerczek-Zięba G. Structure and antioxidant activity of polyphenols derived from propolis. Molecules 2014; 19(1): 78-101.
5. Bankova V, Popova Milena, Bankova BT. Propolis volatile compounds: chemical diversity and biological activity: a review. Chem Cent J. 2014; 8(1): 28, doi: 10.1186/1752-153X-8-28.
6. Demir S, Aliyazicioglu Y, Turan I, Misir S, Mentese A, Yaman Ozer S, *et al.* Antiproliferative and proapoptotic activity of Turkish propolis on human lung cancer cell line. Nutr Cancer. 2015; 68(1): 165-72.
7. Ahn MR, Kumazawa S, Usui Y, Nakamura J, Matsuka M, Zhu F, *et al.* Antioxidant activity and constituents of propolis collected in various areas of China. Food Chem. 2007; 101(4): 1383-92.
8. Yalcin CO, Aliyazicioglu Y, Demir S, Turan I, Bahat Z, Misir S, *et al.* Evaluation of radioprotective effect of Turkish propolis on foreskin fibroblast cells. J Can Res Ther. 2016; 12(2): 990-4.
9. Pereira AD, Andrade SF, Oliveira Swerts MS, Maistro EL. First *in vivo* evaluation of the mutagenic effect of Brazilian green propolis by comet assay and micronucleus test. Food Chem Toxicol. 2008; 46(7): 2580-4.
10. Aliyazicioglu Y, Deger O, Ovali E, Barlak Y, Hosver I, Tekelioglu Y, *et al.* Effects of Turkish pollen and propolis extracts on respiratory burst for K-562 cell lines. Int Immunopharmacol. 2005; 5(11): 1652-7.

11. Burdock GA. Review of the biological properties and toxicity of bee propolis (Propolis). Food Chem Toxicol. 1998; 36(4): 347-63.
12. Abubakar MB, Abdullah WZ, Sulaiman SA, Ang BS. Polyphenols as key players for the antileukaemic effects of propolis. Evid Based Complement Alternat Med. 2014; Article ID: 371730, doi: 10.1155/2014/371730.
13. Mocanu MM, Nagyand P, Szöllosi J. Chemoprevention of breast cancer by dietary polyphenols. Molecules. 2015;20(12):22578-620.
14. Silva-Carvalho R, Baltazar F, Almeida-Aguiar C. Propolis: a complex natural product with a plethora of biological activities that can be explored for drug development. Evid Based Complement Alternat Med. 2015; Article ID: 206439. doi: 10.1155/2015/206439.
15. Ivona Jasprica I, Mornar A, Debeljak Z, Smolcic-Bubalo A, Medic-Saric M, Mayer L, *et al*. *In vivo* study of propolis supplementation effects on antioxidative status and red blood cells. J Ethnopharmacol. 2007;110(3):548-54.
16. Turan I, Deger O, Aliyazicioglu Y, Demir S, Kilinc K, Sumer A. Effects of Turkish propolis on expression of Hogg-1 and Neıl-1. Turk J Med Sci. 2015;45(4):804-11.
17. Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional foods: Impact on human health. Pharmacogn Rev. 2010;4(8):118-26.
18. Slinkard K, Singleton VL. Total phenol analyses: automation and comparison with manual methods. Am J Enol Viticult. 1977; 28(1): 49-55.
19. Chang CC, Yang MH, Wen HM, Chern JC. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J Food Drug Anal. 2002;10(3):178-82.
20. Oyaizu M. Studies on product of browning reaction prepared from glucose amine. Jpn J Nutr. 1986; 44(6): 307-315.
21. Yu L, Haley S, Perret J, Harris M, Wilson J, Qıan M. Free radical scavenging properties of wheat extracts. J Agric Food Chem. 2002;50(6):1619-24.
22. Dinis TCP, Madeira VMC, Almeida LM. Action of phenolic derivates (acetoaminophen, salicylate and 5-aminosalycilate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Arch Biochem Biophys. 1994;315(1):161-9.
23. Kang KA, Lee KH, Zhang R, Pıao M, Chae S, Kim KN, *et al.* Caffeic acid protects hydrogen peroxide induced cell damage in WI-38 human lung fibroblast cells. Biol Pharm Bull. 2006;29(9):1820-4.
24. Watanabe MAE, Amarante MK, Conti BJ, Sforcin JM. Cytotoxic constituents of propolis inducing anticancer effects: a review. J Pharm Pharmacol. 2011;63(11):1378-86.
25. Sforcin JM. Biological properties and therapeutic applications of propolis. Phytother Res. 2016;30(6):894-905.
26. Barlak Y, Deger O, Colak M, Karatayli SC, Bozdayi AM, Yucesan F. Effect of Turkish propolis extracts on proteome of prostate cancer cell line. Proteome Sci. 2011;9(1):74. https://doi.org/10.1186/1477-5956-9-74.
27. Shuai H, Cui-Ping Z, Kai W, George QL, Fu-Liang H. Recent advances in the chemical composition of propolis. Molecules. 2014;19(12):19610-32.
28. Jin D, Mumper RJ. Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. Molecules. 2010;15(10):7313-52.
29. Mihai CM, Mărghitaş LA, Dezmirean DS, Bărnuţiu L. Correlation between polyphenolic profile and antioxidant activity of propolis from Transylvania. Anim Sci Biotechnol. 2011;44(2):100-3.
30. Can Z, Yıldız O, Şahin H, Asadov A, Kolaylı S. Phenolic profile and antioxidant potential of propolis from Azerbaijan. Mellifera 2015;15(1):16-28.
31. Yang H, Dong Y, Du H, Shi H, Peng Y, Li X. Antioxidant compounds from propolis collected in Anhui, China. Molecules. 2011;16(4):3444-55.
32. Socha R, Gałkowska D, Bugaj M, Juszczak L. Phenolic composition and antioxidant activity of propolis from various regions of Poland. Natural Product Res. 2015; 29(5): 416-22.
33. Geckil H, Ateş B, Erdoğan S, Yılmaz I, Durmaz G. Antioxidant, free scavenging and metal chelating characteristics of propolis. Am J Biochem Biotechnol. 2005;1(1):27-31.
34. Siripatrawan U, Vitchayakitti W, Sanguandeekul R. Antioxidant and antimicrobial properties of Thai propolis extracted using ethanol aqueous solution. Int J Food Scı Tech. 2012;48(1):22-7.
35. Wang BJ, Lien YH, Yu ZR. Supercritical fluid extractive fractionation-study of the antioxidant activities of propolis. Food Chem. 2004; 86(2): 237-43.
36. Mavri A, Abramovic H, Polak T, Bertoncelj J, Jamnik P, Moz˘ina SS, *et al*. Chemical properties and antioxidant and antimicrobial activities of Slovenian propolis. Chem Biodivers. 2012; 9(8): 1545-58.
37. Miguel MG, Nunes S, Dandlen SA, Cavaco AM, Antunes MD. Phenols, flavonoids and antioxidant activity of aqueous and methanolic extracts of propolis (Apis mellifera L.) from Algarve, South Portugal. Food Sci Technol (Campinas). 2013; 34(1): 16-23.
38. Aliyazicioglu Y, Demir S, Turan I, Cakiroglu TN, Akalin I, Deger O, *et al*. Preventive and protective effects of Turkish propolison H2O2-induced DNA damage in foreskin fibroblast cell lines. Acta Biol Hung. 2011; 62(4): 388-96.
39. Sharon AA, Nora MOB. Mechanism of protection by the flavonoids, quercetin and rutin, against tert-butylhydroperoxide and menadione induced DNA single strand breaks in Caco-2 cells. Free Radic Biol Med. 2000; 29(6): 507-14.
40. Yen GC, Duh PD, Tsai HL. Antioxidant and pro-oxidant properties of ascorbic acid and gallic acid. Food Chem. 2002; 79(3): 307-13.
41. Murase H, Shimazawa M, Kakino M, Ichihara K, Tsuruma K, Hara H. The effects of Brazilian green propolis against excessive light-induced cell damage in retina and fibroblast cells. Evid Based Complement Alternat Med. 2013; Article ID:238279. doi: 10.1155/2013/238279.
42. Song JJ, Lim HW, Kim K, Kim KM, Cho S, Chae SW. Effect of caffeic acid phenethyl ester (CAPE) on H2O2 induced oxidative and inflammatory responses in human middle ear epithelial cells. Int J Pediatr Otorhinolaryngol. 2012; 76(5): 675-9.
43. Wang X, Stavchansky S, Zhao B, Bynum J, Kerwin SM, Bowman PD. Cytoprotection of human endothelial cells from menadione cytotoxicity by caffeic acid phenethyl ester: The role of heme oxygenase-1. Eur J Pharmacol. 2008; 591(1): 28-35.
44. Dhanya R, Arun KB, Syama HP, Nisha P, Sundaresan A, Santhosh KTR, *et al*. Rutin and quercetin enhance glucose uptake in L6 myotubes underoxidative stress induced by tertiary butyl hydrogen peroxide. Food Chem. 2014; 158: 546-54.
45. Lee KJ, Choi JH, Hwang YP, Chung YC, Jeong HG. Protective effect of caffeic acid phenethyl ester on tert-butyl hydroperoxide-induced oxidative hepatotoxicity and DNA damage. Food Chem Toxicol. 2008;46(7):2445-50.
46. Munari CC, Alves JM, Bastos JK, Tavares DC. Evaluation of the genotoxic and antigenotoxic potential of Baccharis dracunculifolia extract on V79 cells by the comet assay. J Appl Toxicol. 2009; 30(1): 22-8.

SUMMARY

- Propolis is a natural resinous material with antioxidative, antitumoral, antimutagenic, and other beneficial properties.
- EEP has had good antioxidant properties, rich in polyphenolic and flavonoids compounds.
- EEP reduced the amount of intracelular ROS on t-BHP induced oxidative stress in foreskin fibroblast cells.
- Our results suggest that EEP may be a novel candidate for the development of new natural product based therapeutic agents.

PICTORIAL ABSTRACT



About Authors

Sema Misir: Is presently working as Research Assistant in Department of Biochemistry in Cumhuriyet University. She has experience in the area of pharmaceutical chemistry, cancer, natural products, and oxidative stress.

Yuksel Aliyazicioglu: Is presently working as Professor in Department of Medical Biochemistry in Karadeniz Technical University. He has experience in the area of clinical biochemistry, cell culture, cancer, oxidative stress, and molecular biochemistry. He has published a number of papers in his expertise area in various journals.

Selim Demir: Is presently working as Associate Professor in Department of Nutrition and Dietetics in Karadeniz Technical University. He has experience in the area of pharmaceutical chemistry, cell culture, cancer, and molecular biochemistry.

Ibrahim Turan: Is presently working as Assistant Professor in Department of Genetic and Bioengineering in Gumushane University. At the same time he is also working as a manager in the Medicinal Plants, Traditional Medicine Practice and Research Center in Gumushane University. He has experience in the area of pharmaceutical chemistry, genetics, cancer, molecular biology and biochemistry.

Orhan Deger: Is presently working as Professor in Department of Medical Biochemistry in Karadeniz Technical University. He has experience in the area of clinical biochemistry, bee products, cancer, oxidative stress, and molecular biochemistry. He has published a number of papers in his expertise area in various journals.

Cite this article: Misir S, Aliyazicioglu Y, Demir S, Turan I, Yaman SO, Deger O. Antioxidant Properties and Protective Effect of Turkish Propolis on T-BHP-Induced Oxidative Stress in Foreskin Fibroblast Cells. Indian J of Pharmaceutical Education and Research. 2018;52(1):94-100.