NOVEL ANTICANCER COMPOUNDS OF PROPOLIS AGAINST THREE DIFFERENT CANCER TYPES

NİZAMİ DURAN[1], GÜLAY GÜLBOL DURAN[2], DURMUŞ ALPASLAN KAYA[3], KEMAL JENEDI[1], ŞEVKET ÖZTÜRK[3], MADALINA GEORGIANA ALBU[4], ZINA VULUGA[5]

[1] *Mustafa Kemal University, Medical Faculty, Microbiology Dept., Hatay/Turkey*

[2] *Mustafa Kemal University, Health Collage, Medical Biology&Genetics, Hatay/Turkey*

[3] *Mustafa Kemal University, Agriculture Faculty, Field Crops Dept., Hatay/Turkey*

[4] *The National Research & Development Institute for Textiles and Leather, Division Leather and Footwear Research Institute, Collagen Department, Bucharest/Romania*

[5] *INCDCP-ICECHIM 202 Spl. Independentei, 060021, Bucharest, Romania*

Cancer is currently the second most common cause of death. The drugs used for conventional cancer therapies cause unwanted side effects. At best, these kinds of drugs used to treat cancer patients only extend the patient's lifespan by a few years. Therefore new drug research in cancer treatment is continuing rapidly. Natural products have recently been investigated as promising agents for the different types of cancer. Recently, propolis has attracted attention due to its various of pharmacological properties. In this study, we aimed to investigate the chemical charecterization of propolis samples collected from Hatay region. Besides this, the aim of this study was to estimate the proliferative effects of the propolis samples on three different cancer lines (A549; human lung adenocarcinoma, HeLa; human cervical carcinoma, A498 human renal carcinoma). The GC-MS (gas chromatography mass spectrometry) analyses were performed for the analyses of the constituents of the propolis samples. Cell culture. Human cell cultures (A549; human lung adenocarcinoma, HeLa; human cervical carcinoma, A498 human renal carcinoma) were maintained by weekly transfers in RPMI-1640 medium supplemented with 10% fetal calf serum with antibiotics (penicillin; 100 U/ml and streptomycin (100 µg/ml) at at 37 °C in 5% CO2. In this study, it was shown that these three propolis components (benzoic acid, phenylethyl alcohol, 9-octadecenoic acid) have to be remarkable antiproliferative effects against human lung adenocarcinoma (A549), human cervical carcinoma (HeLa) and human renal carcinoma (A498) cells. These components can be served as promising propolis compounds for further new drug development.

Keywords: propolis, anticancer, drug, cancer cells

INTRODUCTION

For many centuries, plants have provided a rich source of therapeutic agents. Currently 25% of prescribed drugs worldwide are still derived from plant sources, showing that plant species are still an important source of new drugs for diseases that continue to lack a cure, such as cancer. The use of natural products has been one of the most successful strategies for the discovery of new medicines; natural products have been used for folk medicine purposes throughout the world for thousands of years (Castaldo and Capasso, 2002; Duran, 2012; Duran *et al.*, 2006; Ferlay *et al.*, 2010; Siegel *et al.*, 2012).

Among these kinds of natural products, propolis has attracted increased interest because of pharmacological activities. Propolis is a natural product derived from plant resins collected by honeybees. It is used by bees as glue, a general-purpose sealer, and as draught-extruder for beehives. Propolis has been used in folk medicine for centuries. It is known that propolis possesses anti-microbial, antioxidative, anti-ulcer and anti-tumor activities (Castaldo and Capasso, 2002). Therefore, propolis has attracted much attention in recent years as a useful or potential substance used in medicine and cosmetics products. Furthermore, it is now extensively used in foods and beverages

with the claim that it can maintain or improve human health. The chemical composition of propolis is quite complicated. More than 300 compounds such as polyphenols, phenolic aldehydes, sequiterpene quinines, coumarins, amino acids, steroids and inorganic compounds have been identified in propolis samples. The chemical composition of propolis is quite complex. Over 300 different compounds such as polyphenols, phenolic aldehydes, terpenes, kinins, coumarins, amino acids, inorganic components and steroids have been identified so far in propolis (Castaldo and Capasso, 2002; Duran, 2012). Propolis has various components such as caffeic acid, caffeic acid phenethyl ester, artepilin C, quercetin, naringenin, resveratrol, galangin, genistein and other are the most promising as antitumor agents. The contents of propolis samples depend on the collecting location, time and plant source. Consequently, biological activities of propolis gathered from different phytogeographical areas and time periods vary greatly (Castaldo and Capasso, 2002; Duran, 2012; Duran *et al.*, 2006).

In our earlier studies, propolis samples collected from Hatay region have been identified antiproliferative activity on cancer cell lines (Duran *et al.*, 2011).

Search for new substances with antiproliferative activity towards cancer cells is very important because of the drug resistance and side effects used in conventional cancer therapy (Luqman and Pezzuto, 2010).

In this study, we aimed to investigate the chemical charecterization of propolis samples collected from Hatay region. Besides this, the aim of this study was to estimate the proliferative effects of the propolis samples on three different cancer lines (A549; human lung adenocarcinoma, HeLa; human cervical carcinoma, A498; human renal carcinoma).

MATERIALS AND METHODS

Preparation of the Cell Culture

To determine the cytotoxicity of propolis for human cells, HEK-293 (human embryonic kidney cells) cell line was used. The cells were cultured in RPMI-1640 medium with 10% (w/v) fetal calf serum. The cells was incubated at 37°C in air with 5% carbon dioxide.

To solve propolis samples, DMSO (Sigma, USA) was selected. Stock solutions of propolis samples were solved in the non-toxic DMSO concentration. For this purpose DMSO concentration of 1% was used. The concentrations of propolis were 10, 25, 50, 100, 200 and 400 µg/ml.

In order to test the effect of the propolis samples on HEK-293 cells, 1×10^5 cells were inoculated to the each well of flat-bottomed plates. Then, cultured for 8 hours at 30°C, and the cells were allowed to grow for an additional 96 hours. All experiments were performed in triplicate. Propolis components were determined to be non-toxic up to 150 µg/ml on HEK-293 cells. Therefore, antiproliferative activity studies were carried out in lower than 200 µg/ml concentration.

MTT Antiproliferative Assay

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method was used to determine the effects of propolis samples on cell proliferation in the A549, HeLa and A498 cell lines. Briefly, $1x10^5$ cells/well were evenly distributed to the flat bottomed plates and the plates incubated overnight at 37 °C. Then, each cancer cell

lines were treated with propolis at concentrations of 5, 10, 20, 40, 60, 80, 100, 150, 200 and 250 µg/ml incubated for 48 hours. Later, the medium in each well was replaced with 20 µl MTT (5 mg/ml in phosphate buffer saline) and incubated at 37 °C for 4 hours. The formazan crystals was dissolved in 100 µl dimethyl sulfoxide and it was read with a microplate reader at a 450 nm wavelength**.** The 50% inhibitory concentration (IC_{50}) was calculated as the concentration of test components that achieved a 50% inhibition of cell viability.

Antiproliferation Tests

To determine the antiproliferative activity of propolis on cancer cell lines, 1×10^5 cells were inoculated into each well of flat-bottomed plates, and were cultured for 8 hrs at 28°C. Later, the inoculated plates were incubated at 37°C up to 96 hours. Propolis components were diluted, whereupon decreasing amounts (100, 80, 60, 40, 20, 10 and 5 µg/ml) were placed per well. It was evaluated at the incubation of the 24, 48, 72 and 96 hours. All experiments were performed in triplicate, and the results were expressed as log number cells per milliliter on the percentage of growth inhibition.

Antiproliferative effects of propolis components were evaluated by viable cell counts. Viable cell count were determined by a conventional hemocytometer and the trypan blue exclusion (Burlesson *et al.*, 1992).

Statistical Analysis

The statistical analyses were performed using Student t-test. The p value <0.05 was considered significant. All statistics in the present study were done using SPSS for Windows, version 17.5 SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

In previous studies it has been demonstrated that some propolis components such as CAPE, caffeic acid, caffeic acid phenylethyl ester have anti-proliferative effects against various cancer types (Chen *et al.*, 1996; Coleman *et al.*, 2008; Counter *et al.*, 1998; Gunduz *et al.*, 2005; Motomura *et al.*, 2008; Motomura *et al.*, 2008). The components of propolis such as benzoic acid, phenylethyl alcohol, 9-octadecenoic acid are the most abundant components in Hatay propolis samples (Duran *et al.*, 2011).

In this study we analyzed the components of propolis samples collected from our region. In addition, we investigated the anti-proliferative properties of rich propolis components such as benzoic acid, phenylethyl alcohol, 9-octadecenoic acid.

We have shown that these three propolis components (benzoic acid, phenylethyl alcohol, 9-octadecenoic acid) have to be remarkable antiproliferative effects against human lung adenocarcinoma (A549), human cervical carcinoma (HeLa) and human renal carcinoma (A498) cells (Figure 1-3).

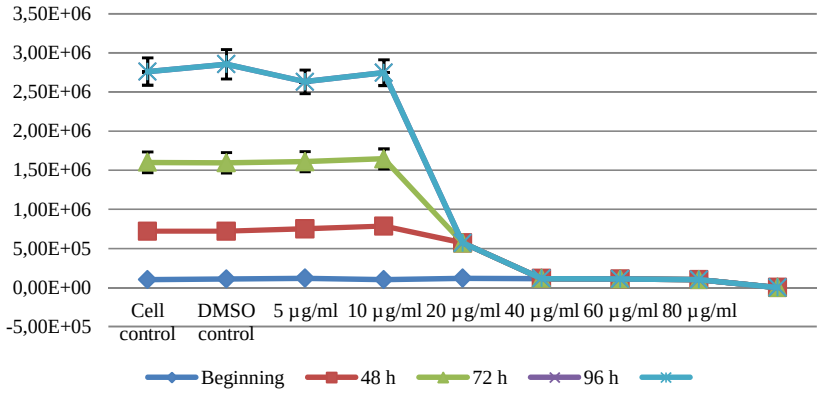

Figure 1. Effects of benzoic acid, phenylethyl alcohol, 9-octadecenoic acid on the proliferation of A549 cells

In the experiments, non-cytotoxic concentrations of the propolis samples was found to be up to 100 µg/ml. Antiproliferative results were given in Figure 1-3. As seen in figures 1 and 2, while propolis components inhibited the A549 and HeLa cell at the concentraton of 20 µg/ml, A-498 cells was found to be inhibited at the concentration of 40 µg/ml propolis (Figure 3).

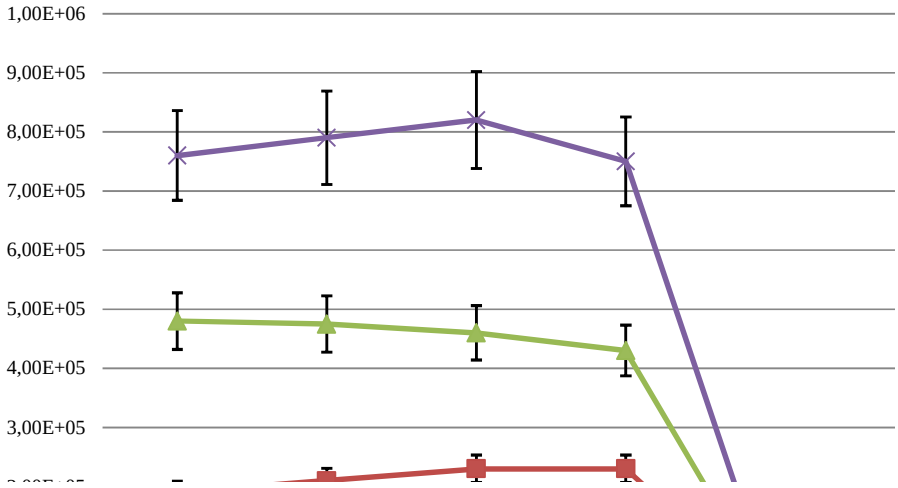

Figure 2. Effects of benzoic acid, phenylethyl alcohol, 9-octadecenoic acid on the proliferation of HeLa cells

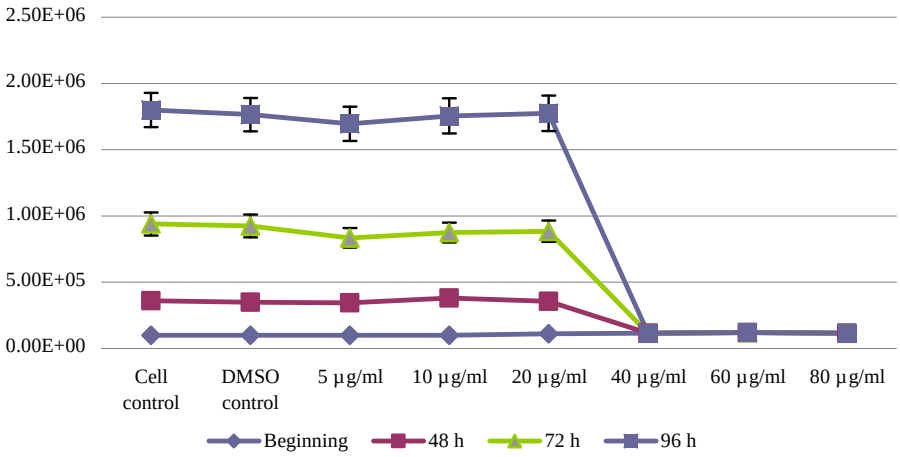

Figure 3. Effects of benzoic acid, phenylethyl alcohol, 9-octadecenoic acid on the proliferation of A-498 cells

CONCLUSIONS

In conclusion, three important components (benzoic acid, phenylethyl alcohol, 9-octadecenoic acid) of propolis samples were found to show an anticancer activity against human lung adenocarcinoma, human cervical carcinoma and human renal carcinoma cells. These components may be a potent drug active substance for these kinds of cancer types. However further researches such as in vivo studies are needed to clarify the effectiveness of these components.

REFERENCES

Castaldo, S. and Capasso, F. (2002), Propolis, an old remedy used in modern medicine. *Fitoterapia*, Suppl 1:S1-6.

Chen, JH., Shao, Y., Huang, MT., Chin, CK. and Ho CT. (1996), Inhibitory effect of caffeic acid phenethyl ester on human leukemia HL-60 cells. *Cancer Lett.*108: 211-214.

Coleman, MP., Quaresma, M., Berrino F, et al. (2008), Cancer survival in five continents: a worldwide population-based study (CONCORD). *Lancet Oncol*. 9(8):730-756.

Counter, CM., Hahn, WC., Wei, W. et al. (1998), Dissociation among in vitro telomerase activity, telomere maintenance, and cellular immortalization. *Proc Natl Acad Sci USA*.95: 14723- 4728.

Duran, N. (2012). Propolis: Implications for Treating *Staphylococcus aureus* Keratitis Propolis: Implications for Treating *Staphylococcus aureus* Keratitis. Beneficial Effects of Propolis on Human Health and Chronic Diseases. Chapter 8. (pp. 133-144). Nova Science Publishers.

Duran, N., Duran, GG., Demir, C., Özer, B., İnci, M. and Yavuz B.(2011), Propolisin HeLa Hücreleri Üzerinde Antineoplastik Etkisinin Araştırılması. 26. Ankem, Antibiyotik ve Kemoterapi Kongresi, P-64, 18-22 Mayıs 2011, Slience Beach Resorts, Kızılağaç-Antalya.

Duran, N., Koc, A., Oksuz, H., Tamer, C., Akaydin, Y., Kozlu, T. and Celik M. (2006), The protective role of topical propolis on experimental keratitis via nitric oxide levels in rabbits. *Mol Cell Biochem*. 281(1-2): 153-161.

Duran, N., Muz, M., Culha, G., Duran, G. and Ozer B, (2011), GC-MS analysis and antileishmanial activities of two Turkish propolis types. *Parasitol Res*, 108:95-105.

Ferlay, J., Shin, HR., Bray, F., Forman, D., Mathers, CD. and Parkin D. (2010), Cancer Incidence and Mortality Worldwide: IARC CancerBase No.10 [Internet]. Lyon, France: International Agency for Research on Cancer. Available from: http://globocan.iarc.fr.

Gunduz, C., Biray, C., Kosova, B. et al. (2005), Evaluation of Manisa propolis effect on leukemia cell line by telomerase activity. *Leuk Res*. 29: 1343-1346.

Luqman, S., Pezzuto, JM. (2010), a promising target for natural products in cancer chemoprevention. *Phytother.Res*., 24: 949-963.

Motomura, M., Kwon, KM., Suh SJ et al. (2008), Propolis induces cell cycle arrest and apoptosis in human leukemic U937 cells through Bcl-2/Bax regulation. *Environ Toxicol Pharmacol*. 26: 61-67.

Siegel, R., Naishadham, D., Jemal, A. (2012), Cancer statistics, *CA Cancer J Clin*. 62(1): 10-29.