Antitumor Activity of Propolis on Differentiated Cancer Cell Lines

Neşe Ersöz Gülçelik¹, Dilara Zeybek², Figen Kaymaz², Ömür Gencay³, Bekir Salih⁴, Esin Asan⁵, Kadriye Sorkun⁶, Aydan Usman¹

¹ Department of Endocrinology and Metabolism, Ankara Numune Training Hospital, Ankara, Turkey
² Department of Histology, Hacettepe Medical School; Ankara, Turkey
³ Department of Biology, Hacettepe Faculty of Science, Ankara, Turkey
⁴ Department of Chemistry, Hacettepe Faculty of Science, Ankara, Turkey

Abstract

Propolis is a natural bee product with several pharmacological activities. Nowadays, it is also investigated for its antitumor properties. There are controversies on the antitumor activity of propolis, not all tumor cells seem to respond to propolis treatment. The aim of our study is to evaluate the activity of propolis on differentiated thyroid cancer cell lines. Trypan blue test and MTT assay were performed to evaluate the cell viability of B-CPAP cells after propolis treatment and compared with propolis free cells and normal thyroid cells. Evaluation with light microscopy revealed after treatment with propolis, cell count decreased and B-CPAP cells displayed morphologic changes with a smaller appearance. Trypan blue exclusion test showed a decrease in cell viability in different concentrations of propolis at 48 and 72 h. Cell viability was measured by MTT after the treatment of B-CPAP cells with propolis at 72 h. The decrease in cell viability was statistically significant only at 0.0001 diluted propolis (p=0.048). The decrease in cell viability in normal thyroid cell line was not statistically significant. In conclusion, propolis has weak antitumor activity on differentiated thyroid cell line which may contribute to different degrees of sensitivity of propolis among cancer cells.

Keywords: Propolis, differentiated thyroid cancer, BCAP

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Corresponding Author: Neşe Ersöz Gülçelik, Department of Endocrinology and Metabolism, Ankara Numune Training Hospital, Ankara, Turkey.
E-mail: neseersoz@hotmail.com
Introduction

Propolis is a sticky resinous bee product produced from a mixture of beeswax and the resin collected from trees, leaf buds and twigs. Propolis exhibits a broad spectrum of biological activities, since it contains a variety of chemical compounds including polyphenols, terpenoids, steroids, amino acids and various inorganic compounds. Propolis is used extensively in traditional medicine for its antioxidant, anti-inflammatory and immunomodulatory effects [1]. It is commercially found as an ingredient of vitamins, droplets, toothpaste, mouthwash, creams and lotions for its antioxidative and antiinflammatory effects. Besides these effects, propolis is searched for its antiproliferative effect on cancer cells. A number of studies addressed its anticancer activity [2-8].

The anticancer effect of propolis is supposed to be via inducing apoptosis and cell cycle arrest. Propolis and its active compounds, mainly CAPE, are shown to inhibit cell cycle proliferation and induce apoptosis in various cancer types [9-11]. The antiproliferative effects are mainly dependent on the dose and the region (collected)?-which effects the chemical composition- of propolis.

Several studies have investigated the differences in chemical compositions of propolis in respect to the type of bee and the region and plants that the resin is collected [12-14]. The effect of propolis from Turkey on breast cancer cell line, prostate cancer cell line and leukemia cell lines were shown previously [6,16-17]. However, propolis may show discrete effects on different cancer cells [4].

In this study, we aimed to evaluate anticancer activity of propolis on differentiated thyroid cancer cell line.

Materials and methods

Cell line and cell culture

The human papillary thyroid cancer cell line B-CPAP was purchased from Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Braunschweig, Germany). The normal thyroid follicular epithelial cell line Nthy-orí 3-1 was obtained from European
Collection of Cell Cultures (ECACC, Wiltshire, UK). Both cell lines were cultured in RPMI 1640 (Sigma Chemical Co.) supplemented with 10% heat-inactivated FCS, 2 mM L-glutamine and 1% penicillin/streptomycin under standard cell culture conditions (37°C, 100% humidity and 5% CO₂).

**Preparation of ethanol extracts of propolis**

Propolis extracts were obtained from Department of Biology, Faculty of Science, Hacettepe University, Ankara, Turkey. First, they were put in a freezer and hardened and then ground in a hand grinder. One hundred grams of each sample were dissolved in 300 ml of 96% ethanol. This mixture was periodically stirred and incubated for 4 weeks at 30°C in a tightly closed bottle. After incubation, the supernatant was filtered twice with Whatman nos. 4 and 1 filterpapers. The final filtered concentrated solution (concentrated EEP) was diluted in 1:10 ratio (w/v) with 96% ethanol to protect it from microbial attack. A portion of the same diluted solution was evaporated to dryness for gas chromatography–mass spectrometry (GC–MS) analysis. Five milligrams of residue was mixed with 75 ml of dry pyridine and 50 ml bis (trimethylsilyl) trifluoroacetamide, heated at 80°C for 20 min and the supernatant was analyzed by GC-MS.

**Determination of cell viability**

The cytotoxic effects and cell viability of propolis were determined by Trypan blue exclusion test and MTT assay. Trypan blue exclusion test was performed on propolis treated cells (at doses 0.0001, 0.00001) incubated for 48 and 72 hours. After the incubation, cells were removed by %0.05 trypsin-EDTA and centrifuged at 800rpm for 5 minutes. 100 µl of suspension and 100 µl %0.4 tyripan blue was mixed and the cells were counted. The percentage of viable cells were calculated as follows: viable cells (%) = (total number of viable cells per ml of aliquot/ total number of cells per ml of aliquot) x100.

MTT assay is based on the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide to formazan. Viable cells convert yellow-colored MTT to violet-colored formazan crystals using the mitochondrial succinate dehydrogenase, which is a part of the respiratory chain. Therefore, cell viability can be determined by measuring the optical density of the formazan product with regard to cellular respiration and metabolic rate. The 3-(4,5-
dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide dye solution (MTT; Applichem, Darmstadt, Germany) was prepared as 5mg/ml in PBS at 37°C. B-CPAP thyroid cell lines were plated 1×10⁴ cells/well in 96-well plates in 100μl of culture medium for 18 hours. When the cultures were at sub-confluence the medium was removed. The cell monolayers were washed with PBS and exposed to 100μl of culture medium (untreated) and to different concentrations of propolis (0.0001 and 0.00001 μl) for 72h. At the end of the treatment, 10μl of MTT dye was added to each well and incubated for 4h at 37°C. After 4 hours 100 μl of isopropanol was added in each well and determined at 570 nm in a microplate reader (Versamax microplate reader).

Results

Evaluation with light microscopy revealed that B-CPAP cells had clear cytoplasms, have arced nucleolus in euchromatic nuclei and showed epitheloid morphology. After treatment with propolis, cell count decreased and B-CPAP cells displayed morphologic changes with a smaller appearance (Figure 1). Tyripan blue exclusion test showed a decrease in cell viability in different concentrations of propolis at 48 and 72 h (Figure 2). Cell viability was measured by MTT after the treatment of B-CPAP cells with propolis at 72 h. Cell viability of propolis-free and propolis-treated B-CPAP cell lines and normal thyroid cell lines are shown in Fig. 3. Cell viability was 100 % in propolis-free B-CPAP cells (control cells) and, 44% and 61% in propolis treated B-CPAP cells at concentrations of 0.0001 and 0.00001, respectively. The decrease in cell viability was statistically significant only at 0.0001 dose (p=0.048). In normal thyroid cell line cell viability was %100 in propolis free control group and 51% and 65% for propolis treated cells at concentrations of 0.0001 and 0.00001, respectively. The decrease in cell viability was not statistically significant.
Figure 1. The number of B-CPAP cells was decreased after propolis treatment. **A**: Untreated B-CPAP cells with round, clear morphologic appearance. **B**: The cell count was decreased and B-CPAP cells displayed a smaller appearance with degenerated morphology after treatment with propolis. (A, B: X200)

![Figure 1](image1.png)

Figure 2. Trypan blue dye exclusion test to show the cell viability. Propolis at 0.0001 concentration decreased the viability of B-CPAP cells at 48 and 72h.

![Figure 2](image2.png)
Figure 3. MTT assay showing the decrease in the cell viability of normal (Nthy-ori 3-1) and papillary (B-CPAP) cell lines after treatment with propolis for 72 h. Propolis caused a concentration dependent decrease in the cell viability of thyroid cells lines. The decrease in cell viability was statistically significant only at 0.0001 dose. Data are expressed as percentage of cell viability with respect to control. Each column represents the mean and S.E.M. of three replicates of two separate experiments (*P<0.05).

Discussion

Propolis is used as an antifungal, antibacterial, antiviral, anti-inflammatory and anticancer agent in traditional medicine. The anticancer activity is of clinical interest because of the need for new anticancer treatment agents. Prominent effect of propolis on cancer cells is its ability to initiate apoptosis in cancer cells [3,11]. Apoptosis is a programmed cell death. There are two main pathways of apoptosis [2]: 1) Extrinsic: external signal induction via receptors of tumor necrosis factor (TNF) such as Fas, TRAIL-R1 and R2; 2) Intrinsic: mediated by mitochondria and pro-apoptotic proteins [2]. Impairment of apoptotic signaling is suggested to be associated with oncogenesis and chemoresistance of transformed cells. Apoptosis plays an important role in chemotherapy-induced killing of tumor cells. Several anti-cancer drugs and natural products used in cancer chemotherapy have apoptosis inducing activity [2,6]. Propolis extracts and its active compounds shown to induce the intrinsic pathway through the
release of cytochrome c from mitochondria to cytosol, through caspase cascade, activation of pro-apoptotic proteins Bax, Bad, p53 and p21 and stimulation of TRIAL and fas receptors [2]. The inductive effect of propolis and its active compounds -mainly CAPE- on apoptosis of various types of cancer cells depends on the concentration of the natural products used.

There are some controversies in antitumor activity of propolis. 1) The chemical composition of propolis changes depending on the region of propolis 2) The antitumor activity is dose dependent 3) Propolis may not always induce apoptosis 4) Antitumor activity may be specific for some cancer cells, not all.

Propolis has different chemical compositions in respect to the type of bee and the region and plants that the resin is collected [12-14]. The anticancer effect of propolis was previously demonstrated in a few studies from the Turkey [6,16,17]. Vatansever et al. demonstrated that antitumor activity of ethanol extract of propolis, which is one of the richest sources of phenolic acids and flavonoids from Turkey, showed apoptosis induction strongly dependent on the concentration and dilutions of EEP on breast cancer cell line MCF-7 [6]. In regards to antitumor activity, dilutions of EEP 0.125 and 0.063mg/ml were more effective (17.5–100%) than dilutions of EEP 0.25 and 0.5 mg/ml (5.11–18.97%). Barlak et al. demonstrated antiproliferative activity of propolis on prostate cancer cell line [17]. Gunduz et al. showed the antitumor and apoptotic effect of Manisa propolis via inhibiting telomerase expression [16].

Propolis may have variable efficiency and effects on different cell types. Although many studies have shown the effect of propolis on inducing the apoptosis, some reported the antiapoptotic activity of propolis. Nadia et al. showed that propolis restored induced permeability transition pore opening in the rat liver mitochondria after exposure to ferulenol [18]. Orsolic and Basic studied the antitumor effect of water-soluble derivatives propolis (WSDP) from Croatia and Brazil on mammary carcinoma cells (MCA), human epithelial carcinoma cell line (HeLa), and Chinese hamster lung fibroblast cells (V79) [5]. Their study showed that the percentage of apoptotic MCA cells increased from 20% (in controls) to 24% and 26% after exposure to 50 μg/ml of Brazilian and Croatian propolis, respectively and the percentage of apoptotic HeLa cells (2% in controls) was 10% for Croatian propolis and 9.5% for Brazilian propolis. However, contradictory results were seen in V79 cells: The percentage of apoptotic V79 cells treated with both Brazilian and Croatian propolis was smaller than in
nontreated cells. These results indicate that propolis may have different degrees of sensitivity among cancer cells and normal fibroblasts.

In our study, we evaluated the anticancer activity of propolis on differentiated thyroid cancer cells. Light microscope, tyrian blue and MTT test demonstrated that antiproliferative activity of propolis is dose dependent. The effective dose of propolis on BCNA cells is 0.0001 at 72h. Lower dose of 0.00001 resulted in a decrease in cell viability but failed to reach a statistical significance. Although propolis resulted in cell death in BCNA cells at 0.0001 concentrations at 72h the statistical significance was not powerful.

In conclusion, the weak antitumor activity of propolis on thyroid cancer cell lines does not seem to be promising. Our results may contribute to the different degrees of sensitivity of propolis among cancer cells. These contradictory results indicate that further studies are needed to establish the role of propolis extracts on cancer cells.

**Disclosure:** There is no conflict of interest.

**References**


