

Original Research Article

Cytotoxic Effect of Turkish Propolis on Liver, Colon, Breast, Cervix and Prostate Cancer Cell Lines

Ibrahim Turan^{1,2*}, Selim Demir³, Sema Misir^{3,4}, Kagan Kilinc¹, Ahmet Mentese⁵, Yuksel Aliyazicioglu⁵ and Orhan Deger⁵

¹Department of Genetic and Bioengineering, Faculty of Engineering and Natural Sciences, ²Medicinal Plants, Traditional Medicine Practice and Research Center, Gumushane University, 29100, Gumushane, ³Department of Medical Biochemistry, Institute of Health Sciences, Karadeniz Technical University, 61080, Trabzon, ⁴Department of Biochemistry, Faculty of Pharmacy, Cumhuriyet University, 58140, Sivas, ⁵Department of Medical Biochemistry, Faculty of Medicine, Karadeniz Technical University, 61080, Trabzon, Turkey

*For correspondence: **Email:** ibrahimtrn@gmail.com; **Tel:** +90 456 233 10 00; **Fax:** +90 456 233 75 67

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Abstract

Purpose: To investigate the total polyphenolic and flavonoid contents, antioxidant power and cytotoxic activity of ethanol extracts of Turkish propolis (EEP).

Methods: The total polyphenolic and flavonoid contents of EEP were determined by spectrometric methods. Antioxidant power and cytotoxic activity of EEP were evaluated using ferric reducing antioxidant power (FRAP) and MTT assays, respectively.

Results: The total polyphenolic and flavonoid contents, and FRAP value of EEP were 124.6 ± 1.5 mg gallic acid/g sample dry weight, 42.0 ± 0.8 mg quercetin/g sample dry weight and 311.0 ± 2.5 mg trolox/g sample dry weight, respectively. EEP exhibited powerful cytotoxic effects against the five human cancer cell lines. The highest cytotoxic activity of Turkish EEP was demonstrated on PC-3 cell line ($IC_{50} = 20.7 \pm 3.4$ μ g/mL).

Conclusion: The results demonstrate that EEP is a good source of antioxidant and a natural antitumor agent capable of reducing cancer cell proliferation.

Keywords: Propolis, Polyphenols, Cytotoxic effect, Cancer cell lines, Antioxidant activity

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INTRODUCTION

Propolis is a natural honeybee product that has long been used in traditional medicine [1]. Honeybees collect propolis from exudates and buds of various plants and mix it with their own salivary secretions and waxes [2]. The composition of propolis varies depending on the climate and geography of the region where it is harvested [1]. Accumulated evidence has demonstrated the presence of more than 300 compounds in different propolis samples [3]. The main chemical groups present in propolis contain

phenolic acids or their esters, flavonoids, stilbenes, β -steroids, terpenes, fatty acids and inorganic compounds [2,4]. Propolis has many biological and pharmacological properties, including antibacterial, anti-cariogenic, anti-inflammatory, anti-oxidative, anti-mutagenic and anti-cancer, among others [2,5,6]. The biological effects of propolis are attributed to its polyphenol content [7]. Propolis is today widely used in medicine, cosmetics and food industries due to its versatile biological and pharmacological activities [2].

Cancer is a universal health problem and the most widespread cause of death. Chemotherapeutic drugs may not be effective against some cancer cells, and the efficacy of such drugs may also decrease due to the development of drug resistance in cancer cells [8]. Researchers have focused on the potential use of natural compounds as chemotherapeutic or complementary agents in the treatment of cancer due to the inefficacy of the drugs that are currently available [9]. Numerous studies have investigated the anticancer activity of propolis from varying regions on different cancer cell lines [4].

The number of studies investigating the anticancer activity of Turkish propolis is limited [10,11]. The purpose of this study is therefore to identify the antioxidant properties and cytotoxic activities of EEP.

EXPERIMENTAL

Chemicals

Dimethylsulfoxide (DMSO), sodium carbonate, folin reagent, gallic acid, ethanol, aluminium nitrate, potassium acetate, quercetin, $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$, $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$, potassium ferricyanide, trichloroacetic acid, iron(III) chloride, trolox, cisplatin, trypan blue, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) from Sigma (St. Louis, MO, USA). Penicillin-streptomycin and trypsin from Gibco (Paisley, England), Eagle's Minimum Essential Medium (EMEM), RPMI-1640 Medium and Kaighn's Modification of Ham's F-12 Medium (F-12K) from Lonza (Verviers, Belgium), fetal bovine serum (FBS) from Biochrom (Berlin, Germany), phosphate buffer saline (PBS) tablet from Medicago (Uppsala, Sweden).

Quercetin (1 mg/mL) and cisplatin (1 mg/mL) were dissolved in DMSO to prepare their stock solutions.

Preparation of propolis extracts

The propolis samples used in this study were produced by honey bees (*Apis mellifera* L) in the region of Trabzon, Turkey, and were provided the by Trabzon Agricultural Development Cooperative. For preparing stock EEP (50,000 µg/mL), 1 g propolis was dissolved in 20 mL absolute ethanol and then incubated at 60 °C and 150 rpm for 24 h. After incubation, the sample was centrifuged at 4000 rpm for 10 minutes. Supernatants were filtered through filter paper and 0.22 µm filters. [12]

Evaluation of total polyphenolic content (TPC)

Total polyphenols in the EEP were determined using Folin-Ciocalteu reagent in a 96-well microplate, as previously described [13]. Gallic acid was used as standard and values were stated as mg gallic acid equivalents (GAE)/g sample dry weight.

Determination of total flavonoid content (TFC)

Total flavonoids in the EEP were evaluated in a 96-well microplate using aluminum nitrate colorimetric method [14]. Quercetin was used as standard and values were stated as mg quercetin equivalents (QE)/g sample dry weight.

Determination of ferric reducing antioxidant power (FRAP)

The ferric reducing antioxidant power of EEP was determined using the method based on ferric to ferrous ion reduction at low pH [7]. Trolox was used as a standard, and values were expressed as mg trolox equivalents (TE)/g sample dry weight.

Cell culture

Prostate adenocarcinoma (PC-3), hepatocellular carcinoma (HepG2), colon adenocarcinoma (WiDr), cervix adenocarcinoma (HeLa) and mammary adenocarcinoma (MCF-7) human cancer cell lines were obtained from the America Type Culture Collection (ATCC, USA).

WiDr, MCF-7 and HeLa cells were maintained in EMEM. PC-3 cells in F-12K medium, and HepG2 cells in RPMI-1640 medium. All the media contained L-glutamine, 10 % FBS, 1 % penicillin and streptomycin and the cells were grown in T-75 flasks, with 5 % CO_2 supply at 37 °C in an incubator. The cells were passaged when they reached 70 – 80 % growth in flasks.

Cytotoxicity studies

EEP, quercetin (one of the major flavonoids in EEP) and cisplatin (positive control) cytotoxicity were tested on five human cancer cell lines. Cell viability was determined as previously described [15]. All cells were plated at 5×10^3 cells/well in 96-well cell culture plates and allowed to adhere for 24 h at 37 °C. At the end of 24 h, cells were treated with different concentrations of EEP (0-200 µg/mL), quercetin (0-25 µg/mL) and cisplatin (0-10 µg/mL). The final concentrations of ethanol and DMSO in the medium did not exceed 1 % and these concentrations of ethanol and DMSO

were not harmful to cell viabilities and morphologies. After 72 h incubation 190 μ L medium and 10 μ L MTT dye and a final concentration of 0.25 mg/mL were added to wells and cells were incubated for 2 h. After incubation, well contents were removed and 200 μ L of DMSO was added to all wells and incubated for 60 min. Absorbance was measured using a microplate reader (Versamax, Molecular Devices, USA, California) at 570 nm. All absorbance were compared to control samples (cells without any test compound) which represented 100 % viability. Cell viability was determined as in Eq 1 [16].

$$\text{Cell inhibition (\%)} = [\text{As}/\text{Ac}] \times 100 \quad \dots (1)$$

where As and Ac are the absorbance of the sample and control, respectively. From the plot of log-concentration versus cell viability, half-maximal inhibitory concentration (IC_{50}) values were determined.

RESULTS

TPC, TFC and FRAP values of EEP were found 124.6 ± 1.5 mg GAE, 42.1 ± 0.8 mg QE, and 311 ± 2.5 mg TE per to g sample dry weight, respectively (mean \pm SD, $n=3$). The cytotoxicity results, expressed as IC_{50} , are listed in Table 1. The results indicate that the highest cytotoxic effect of EEP was exerted on PC-3 cell line ($\text{IC}_{50} = 20.7 \pm 3.4$ μ g/mL).

DISCUSSION

There is considerable interest in the anti-proliferative properties of natural products, because these are believed to be relatively non-toxic and have been used as traditional medicines for hundreds of years worldwide [9]. Nowadays, over 70 % of anticancer agents are derived from natural products [17]. Propolis is a natural bee product widely used in traditional medicine for the treatment of various illnesses since ancient times [18]. Today, propolis is consumed as an extract due to its complex resinous structure. The type of solvent and extraction procedures employed therefore further

affect its composition and biological effects [19]. In addition, several studies [20,21] have confirmed that different compounds may be found in propolis content, depending on varieties of the plants and geographical areas from which the resin is collected and the races of bees involved.

Several methods have been used for the extraction of active components from propolis. These methods are maceration, ultrasonic (sonication), soxhlet and microwave extraction. In maceration technique, organic solvents are used to dissolve the components directly without producing heat, so this technique is suitable for heat labile and heat stable substances. Many solvents (water, ethanol, methanol, ethyl acetate, dimethyl sulfoxide, hexane and acetone) have been used in preparing propolis extracts [22]. The most common formulation of propolis in traditional medicine is the ethanol extract [23]. The ethanol extract of Turkish propolis was therefore prepared using maceration technique.

The determination of TPC and TFC are important in various natural products. Physicochemical methods are frequently used for evaluating antioxidant capacities of propolis samples from various different regions since these are practicable, rapid and cheap assays [24]. In our study TPC of EEP was measured at 124.6 ± 1.5 mg GAE/g sample dry weight using the Folin-Ciocalteu method. TPC in ethanolic extracts of propolis have been reported at 174.7 mg GAE/g sample dry weight from China [25], 151.5 mg GAE/g sample dry weight from Brazil [1] and 31.2 - 299 mg GAE/g sample dry weight from other parts of the world [24]. TFC of EEP in this study was 42 ± 0.8 QE/g sample dry weight. TFC in ethanolic extracts of propolis of 45.1 mg QE/g sample dry weight have been reported from China [25] and 2.5 - 176 mg QE/g sample dry weight from other parts of the world [24]. The FRAP method was used to determine total antioxidant capacities of a compound. This is considered a good indicator for total antioxidant power [26].

Table 1: Cytotoxic activity (IC_{50} , μ g/mL)* of EEP and other test compounds

Test compound	HepG2	WiDr	PC-3	HeLa	MCF-7
EEP	27.0 \pm 0.8	62.2 \pm 2.2	20.7 \pm 1.3	36.0 \pm 0.7	28.0 \pm 2.0
Quercetin	8.3 \pm 0.5	8.0 \pm 0.1	4.0 \pm 0.04	1.6 \pm 0.08	8.8 \pm 0.15
Cisplatin	2.4 \pm 0.2	0.99 \pm 0.06	0.54 \pm 0.02	0.66 \pm 0.02	0.41 \pm 0.04

*Mean \pm SD, $n = 3$

In the present study, FRAP value of EEP was 311 ± 2.5 mg TE/g sample dry weight. FRAP values of ethanol extracts of propolis from different regions of Iran are in the range 31.5 - 1650 mg TE/g sample dry weight [14]. The results from the present study are thus largely in agreement with those of other studies; small differences may have arisen due to different methods of propolis extraction, geographic region, harvest season and races of honeybee involved.

Many studies have reported antiproliferative activity of both propolis and various bioactive compounds from propolis [4,21]. The effectiveness of anti-cancer therapy is evaluated by the ability to initiate apoptosis or cell cycle arrest in cancer cells [27]. Apoptosis induction and cell cycle arrest are recommended as main mechanisms of the anticancer activities of propolis [21].

In the present study, we demonstrated the cytotoxic effects of Turkish EEP on five human tumoral cell lines. There are few previous studies of the cytotoxic effects of propolis against human tumoral cell lines. EEP exhibited powerful antiproliferative effects against all studied human cancer cell lines investigated, and the IC_{50} values ranged from 20.7 ± 1.3 to 62.2 ± 2.2 $\mu\text{g/mL}$. Vatanserver *et al* demonstrated that ethanol extracts of Turkish propolis have dose-dependent antiproliferative effects on the MCF-7 cell line [11].

One recent study similarly reported that propolis has a dose- and time-dependent cytotoxic effect against HeLa cells [28]. Szliszka *et al* demonstrated that EEP (5 - 50 $\mu\text{g/mL}$) induces apoptosis in HeLa cell line in a dose dependent manner [29]. Cytotoxic activities of extracts of propolis from different regions against various human cancer cell lines have been reported in the literature [30,31]. Another study has investigated the toxicities of ethanolic extracts of Thai propolis prepared using the maceration technique on the A549 and HeLa cell lines. Thai EEPs exhibit anti-proliferative effects against A549 and HeLa cells and their IC_{50} values have been calculated at 85.05 and 79.83 $\mu\text{g/mL}$, respectively [22]. These results show that Turkish propolis is a good natural product due to its antioxidative and cytotoxic activities among the various types of propolis across the world.

Our data show that IC_{50} values of EEP were higher than those of quercetin. The cytotoxic effect of propolis on cancer cell lines may not therefore derive from quercetin alone, and this result may explain the synergistic effect of all

propolis constituents. Additionally some phenolic compounds of propolis (caffeic acid phenethyl ester (CAPE), quercetin, chrysin) have been investigated in terms of antiproliferative effects [21].

Polyphenolic compounds found in propolis are known to exhibit antioxidant activities, and these activities may play a pivotal role in the cytotoxic effect. The solubilities of Turkish propolis in different solvents have previously been investigated, and ethanol extracts of Turkish propolis was found to have high antioxidant capacities and high quercetin levels [32]. Erdoğan *et al* observed content of Anatolian propolis and reported that it is rich in phenolic compounds, such as caffeic acid, gallic acid, p-coumaric acid, chlorogenic acid, myricetin, catechine and luteolin [33]. Uzel *et al* evaluated chemical compositions and antimicrobial activities of different Anatolian propolis samples. They found that the ethanol extract of propolis samples from Trabzon was rich in flavanones (pinocembrin, naringenin, chrysin) and flavonones (pinobanksin, quercetin, galangine, apigenin and their derivatives) [34].

CONCLUSION

Although much is known about the cytotoxic effects of propolis from different regions, there have been few studies of Turkish propolis. It is probable that the constituents of propolis are responsible for its anti-proliferative activities due to its phenolic content; although these were not isolated in this study, the present work provides a new perspective for further research. Further investigations are required to clarify the molecular mechanism(s) involved in its anticancer effects and to identify individual constituents of Turkish propolis.

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