ANTIPROLIFERATIVE EFFECTS OF TANACETI PARTHENI, HYPERICUM PERFORATUM AND PROPOLIS ON HELA CELLS

DESANKA CENIĆ-MILOŠEVIĆ1, ZORAN TAMBUR1,2, S. IVANČAJIĆ1, TATJANA STANOJKOVIĆ3, NADA GROZDANIĆ3, Z. KULIŠIĆ4 and ZORICA JURANIĆ3

1 Faculty of Stomatology in Pančevo, Pančevo, Serbia
2 Military Medical Academy, 11000 Belgrade, Serbia
3 Institute of Oncology and Radiology of Serbia, 11000 Belgrade, Serbia
4 Faculty of Veterinary Medicine, University of Belgrade, 11000 Belgrade, Serbia

Abstract - Tanaceti partheni, Hypericum perforatum and propolis have been widely used for centuries and are well-documented medicinal plants and natural product. In this study, we investigated the antiproliferative effects of water extracts of ethanolic dry extracts of two different medicinal plants (Tanaceti partheni and Hypericum perforatum) and propolis on HeLa cells. The Tanaceti partheni extract exhibited mild cytotoxic activity. The IC50 was 153.71 µg/mL. The extract of Hypericum perforatum did not show active cytotoxic activity against HeLa cells (IC50 >200 μg/mL). Regarding the antiproliferative effects of Hypericum perforatum, our results are not in correlation with the results of other authors, probably because different Hypericum species and different human cancer cell lines were used. The extract of propolis did not show active cytotoxic activity against HeLa cells (IC50 = 1.08 ± 0.01 mg/mL). The weak antiproliferative effect of propolis on HeLa cells is either due to the use of a low concentration of propolis extracted in weakly polar solvents, or the use of propolis collected in the autumn.

Key words: Antiproliferative effect, Tanaceti partheni, Hypericum perforatum, propolis.

INTRODUCTION

Cancer is a major public health problem worldwide. In 2009, approximately 1 470 000 new cases in the United States have been diagnosed and more than 560 000 people died from cancers (Jemal et al., 2009). Cancer is the second leading cause of death in many countries, as it is in Serbia. Lifestyle and/or environmental factors, especially dietary factors, play regulatory roles in the formation of cancer (Zi-Luan et al., 2011). The major causes are smoking, dietary imbalances, hormones and chronic infections leading to chronic inflammation (Ames et al., 1995). The estimated worldwide incidence of different carcinomas is about 10 million; half of which are in developing countries (Cozzi et al., 2004). Chemotherapy, as a major treatment modality used for the control of advanced stages of malignancies and as a prophylactic against possible metastasis, exhibits severe toxicity on normal tissues (Pandey and Madhuri, 2006). Among cancer patients in the USA, the use of complementary and alternative medicine ranges between 30-75% (Richardson, 2001). This in turn justifies the interest in the search for possible anticancer agents from the flora of different countries.

Over the past decade, herbal medicines have been accepted universally, and they have an impact
on both world health and international trade. Hence, medicinal plants continue to play an important role in the healthcare systems of a large number of the world’s populations (Akerere, 1988). Traditional medicine is widely used in Serbia as well.

The use of medicinal plants and propolis are becoming increasingly appreciated in suppressing cancer growth (Orsolic and Basic, 2003; Waladkhani and Clemens, 1998) and cancer prevention (Pan and Ho, 2008). *Tanacetum parthenium*, *Hypericum perforatum* and propolis have been widely used and well-documented medicinal plants and natural product for centuries (Hansel and Sticher, 2002; Weiss, 1988). Antifungal (Decosterd et al., 1986), antibacterial (Ishiguro et al., 1986), antiviral (Jacobson et al., 2001) and anticancer (Jayasuriya et al., 1989) compounds have been isolated from *Hypericum species*. Propolis has been used in folk medicine from ancient times and has recently become a subject of special interest in the area of oncology research as a source of valuable compounds for the prevention and cancer treatment (Galvao et al., 2007).

In this study, we investigated the antiproliferative effects of water extracts of ethanolic dry extracts of two different medicinal plants (*Tanacetum parthenium* and *Hypericum perforatum*) and propolis on cell lines derived from human cervix adenocarcinoma (HeLa) cells.

**MATERIALS AND METHODS**

**Materials**

*Tanacetum parthenium* was obtained from the area of Pančevo in Serbia, *Hypericum perforatum* from free-growing plants in central Serbia and crude propolis was collected from one bee colony (in the region of Raška) during one time-frame (autumn), to maintain the homogeneity of the raw material.

**Preparation of extracts**

The first extraction was done in a percolator using 70% ethanol. Low-pressure evaporation of the extract was done following extraction. A 2l glass percolator was first lined with cotton wool. Subsequently, it was filled with precut and sifted plant and covered with 70% ethanol. The content was left to macerate for at least 16 h. Following maceration, the extract was poured out of the percolator at the speed of 2 l/h. The amount of poured extract was six times the volume of the starting material (1:6 extract). The extract was then stored for the next 3-5 days, filtered through a series of Whatman-filters and finally passed through a 0.22 μm filter (Millipore, Billerica, MA). Subsequently, the extract was evaporated in a rotational vacuum evaporator until a dry powder was obtained. The temperature in the evaporator was kept below 65°C under a pressure of 15-25 mbar.

Propolis was extracted by the addition of 500 ml of ethanol to 150 g of propolis. The extraction process lasted for 48 h, after which 465 ml of filtrate was obtained.

In the order to obtain water-soluble components, a second extraction was done. The dry ethanolic extracts were weighed and mixed with physiological saline to 10 mg/mL. These suspensions were put in the dark at room temperature for 24 h; they were shaken for 3 h. The supernatants were filtered through 0.22 μm filter paper. The obtained water extracts were used as stock solutions and were diluted with nutrient medium to the various working concentrations.

**Cell line**

Human cervix adenocarcinoma HeLa cells were obtained from the American Type Culture Collection (ATCC) (Manassas, VA, USA). HeLa cells were maintained in the recommended nutrition medium: RPMI 1640 medium supplemented with 100 g/L heat-inactivated (56°C) fetal bovine serum (FBS), 3 mmol/L L-glutamine, 100 g/mL streptomycin, 100 IU/mL penicillin and 25 mmol/L L-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) and adjusted to pH 7.2 with bicarbonate solution. Cells were grown in a humidified atmosphere of 5% CO₂ in air at 37°C.
Treatment of the cell line

HeLa (2 000 cells per well) cells were seeded into 96-well microtiter plates; 24 h later, after the cell adherence, five different double-diluted concentrations of water extracts were added to the wells. The final concentrations of extracts applied to cells were 12.5, 25, 50, 100 and 200 μg/ml; 50% propolis dissolved in 1% ethanol was finally diluted with nutrient medium 120 times and applied to target cells in final concentrations of 0.5, 1, 2 and 4 mg/ml. In the control wells, only the nutrient medium was added to the cells. The cultures were incubated for 72 h.

Determination of cell survival

The effect of extracts on cell survival was determined by the microculture tetrazolium test (MTT) according to Mosmann (1983) with modification by Ohno and Abe (1991), 72 h after addition of the compounds. Briefly, 20 μL of MTT solution (5 mg/ml phosphate-buffered saline) was added to each well. Samples were incubated for a further 4 h under the same conditions. Then 100 μl of 100 g/l sodium dodecyl sulfate was added to dissolve formazan, the product from the conversion of MTT dye by viable cells. The number of viable cells in each well was proportional to the intensity of absorbance, read in an ELISA plate reader at 570 nm 24 h later. To determine cell survival (%), the A of a sample with cells grown in the presence of various concentrations of the investigated extracts was divided by the control optical density (the A of control cells grown only in nutrient medium) and multiplied by 100. IC50 is the concentration of an agent that inhibits cell survival by 50%. The values of IC50 are means from two measurements, each done in triplicate. IC50 were established from dose-dependent data with using Graphpad Prism Ver 3.0 software.

RESULTS

In vitro antitumor activity

The cytotoxic action of Tanacetum parthenium, Hypericum perforatum and propolis extracts were tested on HeLa cells. The IC50 values of the studied extracts are presented in Table 1, while Fig. 1 depicts the cytotoxic curves from MTT assay showing the survival of HeLa cell grown for 72 h in the presence of increasing concentrations of extracts. The Tanacetum parthenium extract exhibited mild cytotoxic activity. The IC50 on HeLa cell lines was 153.71 μg/ml. The extracts of Hypericum perforatum and propolis did not show active cytotoxic activity against the HeLa cell lines (IC50 > 200 μg/ml; IC50 = 1.08 ± 0.01 mg/ml, respectively).

Light microscopy

Results of microscopic examination (Carl Zeiss inverted microscopy, with total magnification 630) of investigated HeLa cells after 72-h treatment with Tanacetum parthenium, Hypericum perforatum and propolis extracts are shown in Fig. 2. Extracts at a concentration of 200 μg/ml (2.0 mg/ml) induced rounding, detachment and decreased the number of HeLa cells, as compared to control cells.

DISCUSSION

In this study, the antiproliferative effects of ethanolic dry extracts of two different medicinal plants, Tanacetum parthenium and Hypericum perforatum, and 1% ethanolic extract of propolis on HeLa cells were investigated.

Tanacetum parthenium (feverfew) was native to Eurasia; specifically the Balkan Peninsula, Anatolia and the Caucasus, but cultivation has spread it around the world and it is now found in Europe, the Mediterranean, North America and Chile (Jeffrey, 2001). The plant has been used as herbal treatment to reduce fever and to treat headaches, arthritis and digestive problems; scientific evidence does not support anything beyond a placebo effect (Pareek et al., 2009). The active ingredients in Tanacetum parthenium include parthenolide and tanetin. There has been some scientific interest in parthenolide, which has been shown to induce apoptosis in some cancer cell lines in vitro and potentially to target cancer stem cells (Guzman and Jordan, 2005; Guzman et al., 2005; Lesiak et al., 2010). Our results showed that Tanacetum
Table 1. Concentrations of extracts that induced a 50% decrease in HeLa cell survival

<table>
<thead>
<tr>
<th>Extracts</th>
<th>HeLa IC50*</th>
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<tr>
<td>Tanaceti partheni</td>
<td>153.71 ± 0.02 μg/ml</td>
</tr>
<tr>
<td>Hypericum perforatum</td>
<td>&gt; 200 μg/ml</td>
</tr>
<tr>
<td>Propolis</td>
<td>1.08 ± 0.01 mg/ml</td>
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Note: *IC50 values were obtained from the filtered extract suspensions. IC50 values were expressed as the mean ± SD determined from the results of MTT assay in three independent experiments.

Fig. 1. (A, B). Representative graph of HeLa cells survival after 72 h cell growth in the presence of increasing concentrations of investigated extracts.
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Parthenolide extract at a concentration of about 153µg/ml (153.71 ± 0.02) inhibited HeLa cell survival by 50% compared with a vehicle-treated control. The percent of parthenolide was 0.78% in the extract of Tanaceti partheni used. (The percentage of parthenolide was determined by quantitative HPLC analysis.

Hypericum is widely known as herbal treatment for depression and it may be useful in treating infected wounds and inflammatory skin diseases (Reutera et al., 2008). A major constituent chemical, hyperforin, may be useful in the treatment of alcoholism, although dosage, safety and efficacy have not been studied (Kumar et al., 2006; Reutera et al., 2008). Hyperforin has also displayed antibacterial properties against Gram-negative bacteria, although dosage, safety and efficacy have not been studied (Cecchini et al., 2007). Traditional medicine has also employed lipophilic extracts from Hypericum as a topical remedy for wounds, abrasions, burns and muscle pain (Reutera et al., 2008). The positive effects that have been observed are generally attributed to hyperforin due to its possible antibacterial and anti-inflammatory effects (Reutera et al., 2008). For this reason, hyperforin may be useful in the treatment of infected wounds and inflammatory skin diseases (Reutera et al., 2008). Hypericum contains various polyphenols: flavonoids (epigallocatechin, rutin, hyperoside, isoquercetin, quercitrin, quercetin, I3, II8-biapigenin, amentoflavone, astilbin, miquelianin) (Yun et al., 2009), phenolic acids (chlorogenic acid, 3-O-coumaroylquinic acid) and various naphtodianthrones (hypericin, pseudohypericin, protohypericin, protopseudohypericin), phloroglucinols (hyperforin, adhyperforin). The naphthodianthrones, hypericin and pseudohypericin along with the phloroglucinol derivative hyperforin are thought to be active components (Umek et al., 1999; Schwob et al., 2002; Tatsis et al., 2007). The percentage content of hypericin in the used extract of Hypericum perforatum was 0.31%. Hypericin complexes are characterized by red staining, which can be used directly for quantitative photometric determination (described in Deutsche Arzneimittel Codex-DAC).

Fig. 2. Light microscopy of HeLa cells cultured with or without the 200µg/ml of extracts: A) control; B) Tanaceti partheni; C) Hypericum perforatum and D) Propolis (2.0 mg/ml of extract) as described in Materials and Methods and photographed 72 h after addition of drugs (Magnification 12.5X, 1.6X, 6.3/0.2).
Hypericum perforatum at concentrations greater than 200µg/ml inhibited HeLa cell survival by 50% compared with a vehicle-treated control. These results are not in correlation with the results of the other authors regarding the antiproliferative effects of Hypericum. Ferraz et al. (2005) evaluated the cytotoxic activity of the crude methanolic extracts of six Hypericum species. Antiproliferative assays using HT-29 human colon carcinoma cells and H-460 non-small cell lung carcinoma were performed. However, the authors found that the most active crude extracts (100 µg/ml) were those of Hypericum caprifoliatum, Hypericum myrianthum and to a lesser extent Hypericum connatum. The differences between their results and ours are probably due to the use of different Hypericum species and different human cancer cell lines.

Propolis is a natural, resinous hive product that honeybees manufacture by mixing their own wax and salivated secretions with resins collected from cracks in the bark of trees and leaf buds (Burdock, 1998; Banskota et al., 2001; Santos et al., 2003; Nakajima et al., 2009). The chemical composition of propolis depends on the vegetation, climate, season and environmental conditions of the area from where it was collected (Santos et al., 2003; Viuda-Martos et al., 2008). It is mainly composed of resin and vegetable balsam (50%), wax (30%), essential and aromatic oils (10%), pollen (5%), and various other substances, including organic compounds and minerals (5%) (Burdock, 1998; Viuda-Martos et al., 2008; Tylkowski et al., 2010). Organic compounds that are identified in different propolis samples are fatty and phenolic acids and esters, substituted phenolic esters, flavonoids (flavones, flavanones, dihydroflavonols, chalcones), terpenes, β-steroids, aromatic aldehydes and alcohols, sesquiterpenes, naphthalene and stilbene derivatives (Marcucci et al., 2001; Valente et al., 2011). Propolis has a long history of use in folk medicine as an antimicrobial, antitumor, antibacterial, antifungal, antiviral, anti-inflammatory, anti-oxidant, anticancer, antiprotozoan, cariostatic, hepatoprotective and immunostimulant natural product (Banskota et al., 2001; Santos et al., 2003; Sforcin, 2007; Ivancajic et al., 2010; Valente et al., 2011). Numerous studies have been conducted to investigate the antiproliferative effects of propolis. In vitro cytotoxicity against human fibrosarcoma, human lung carcinoma, and murine colon carcinoma cells has been demonstrated by propolis and attributed to its benzofuran derivatives (Banskota et al., 1998; Banskota et al., 2000a; Banskota et al., 2000b, Hirota et al., 2000). Other constituents such as artepillin C and diethyl ether have demonstrated cytostatic activity against myeloid cell lines (Hladon et al., 1980; Kimoto et al., 2001; Ahn et al., 2007). Significant results have been seen against T-cell lines (Kimoto et al., 2001). Furthermore, Takara et al. (2007) investigated that in HeLa cells the sensitivity to paclitaxel and doxorubicin, substrates of MDR1, was unchanged in the presence of propolis, while in HeLa/TXL cells, propolis increased sensitivity to these MDR1 substrates. The authors suggested that the extract inhibited the function of MDR1 and increased the sensitivity to MDR1 substrates in HeLa/TXL cells. According to results of this study, the ethanolic extract of propolis collected from one bee colony (region of Raška) during one time-frame (autumn), at a concentration of 1 mg/ml (1.08±0.01) inhibited HeLa cell survival by 50%. The weak antiproliferative effect of propolis on HeLa cells is perhaps a consequence of using a low concentration of propolis extracted in weakly polar solvents or because the propolis was collected in the autumn.

CONCLUSIONS

The extract of Tanaceti partheni exhibited mild cytotoxic activity. The extracts of Hypericum perforatum and propolis did not show active cytotoxic activity against the HeLa cell lines.

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