Morphological evaluation of MDA-MB-231 human breast cancer cells treated with DMEM extract of Turkish propolis

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INTRODUCTION

Among all forms of cancers, breast cancer is the most common and one of the leading causes of death in women. Lifestyle flaws and environmental pollution have raised the prevalence of breast cancer [1-3]. Natural compounds like dietary phytochemicals are used for chemoprevention and complementary therapy for cancer [4,5]. Propolis or bee glue is one of the bee products from Apis Mellifera. It has been used in traditional medicine and apitherapy since ancient times in the treatment of various conditions such as throat and stomach ulcer, wounds, tuberculosis, eczemas, myalgia, rheumatism, oral mucositis, ulcerative colitis,
Turkey propolis (TP) is composed of various compounds such as pinocembrin, pinobanksin, galangin, quercetin, apigenin, naringenin, chicoric, cinnamic, ferulic and caffeic acids and their esters; chrysin, aromatic acids and diterpenic acids [9-11].

Studies have shown that aqueous and ethanol extracts of propolis, and propolis-derived compounds such as quercetin, galangin and kaempferol possess apoptotic effects, antioxidant activities, cytotoxic properties, antiproliferative and anti-inflammatory activities, as well as anti-immunogenic and anti-genotoxic properties in various cancer cell lines [6,10,12].

In general, ethanol and water were popular solvents previously used for extraction of propolis. However, some researchers have also used methanol, n-butanol, dimethyl sulfoxide (DMSO), olive oil, β-cyclodextrin, petroleum ether, polyethylene glycol and hexane as solvents for the extraction process [13-16]. This study was carried out to identify, for the first time, the effects of DMEM extract of propolis on proliferation, cytotoxicity and morphology of MDA-MB-231 cells, relative to control cells.

**EXPERIMENTAL**

**Reagents**

Fetal bovine serum (FBS), penicillin-streptomycin, glycine, DMEM/Ham’s F12 containing or devoid of L-glutamine, phenol red, DMSO, ethylenediaminetetraacetic acid (EDTA), trypsin, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT), trypan blue and NaCl were products of Sigma, UK.

**Sample collection**

The TP used was obtained from Fanus Food Co., Trabzon. The origin plants of Turkey propolis are *Populus sp.*, *Eucalyptus sp.* and *Castanea sativa* [17,18].

**Extraction of TP**

The TP was ground, and 5 g of the ground propolis was dissolved in 20 mL of pure DMEM, with constant stirring for 24 h in a water bath maintained at a temperature of 60 degrees Celsius.

Stock DMEM extract of propolis was obtained after centrifuging for 10 min at 4000 rpm. After collecting the supernatant, microfilters were used to filter and sterilize the extract. The sterilized stock extract was kept at 4 °C away from light. Prior to use, the stock was diluted with DMEM.

**Cell culture**

The MDA-MB-231 cells were purchased from ATCC, and were cultured in DMEM containing L-glutamine (4 mM) and 5 % fetal bovine serum at 37 °C in a humidified atmosphere containing 5 % CO₂. Trypsin (0.25 %) and 0.02 % EDTA solutions were used to carry out passage of the MDA-MB-231 cells [19].

**Cell viability assay**

The effect of the TP extract on MDA-MB-231 cell viability was determined using trypan blue assay. Surviving and dead cells were enumerated under an inverted microscope from 30 field views selected without bias [20]. The results for control and treated MDA-MB-231 cells were determined in triplicate assays.

**MTT assay**

The cells were seeded overnight at a density of 1.5×10⁴ cells/well in 24-well plates, after which they were exposed to the DMEM extract of TP at a dose of 2.5 mg/mL for 72 h. Untreated cells served as control. Proliferation of control and treated cells were assessed colorimetrically using MTT as described earlier [21].

**Morphometric measurements**

Morphological measurements were performed on control MDA-MB-231 cells and treated MDA-MB-231 cells with 2.5 mg/mL DMEM extract of propolis, using a Zeiss inverted microscope hooked to a TV monitor [22]. Treated and control MDA-MB-231 cells chosen at random were categorized as monopolar, bipolar or multipolar; and their diameters, lengths and thicknesses were measured with electronic caliper. The morphometric parameters were measured three times separately [22].
Statistical analysis

Data are presented as mean ± standard error mean (SEM). The results of control and treated cells were compared using Student’s t-test. Statistical significance was assumed at $p < 0.05$.

RESULTS

The DMEM extract of Turkish propolis at a concentration of 2.5 mg/mL reduced relative cell number of the treated cancer cells, albeit insignificantly, as shown Table 1. In addition, they did not decrease percentage cell viability in MDA-MB-231 cells. Process length (PL) field diameter of treated MDA-MB-231 cells decreased only in monopolar cell type, but increased in both bipolar and multipolar cell types, relative to control cells, albeit insignificantly, as shown in Figures 2 and 3, and Table 2. Process thickness value of treated MDA-MB-231 cells was higher than that of control cells in all cell types, but the differences were insignificant (Figure 4 and Table 2). Cell body diameter of treated MDA-MB 231 cells decreased only in monopolar cell type, but increased in bipolar, multipolar and non-process bearing cell types, albeit insignificantly, as shown Figure 5 and Table 2.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Propolis extract concentration (mg/mL)</th>
<th>2.5</th>
<th>0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relative cell number</td>
<td></td>
<td>4.81 ± 0.24</td>
<td>5.50 ± 0.04</td>
</tr>
<tr>
<td>Cell viability (%)</td>
<td></td>
<td>98.74 ± 0.23</td>
<td>98.79 ± 0.10</td>
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</table>
The current study is the first investigation in which the influence of DMEM extract of TP on the morphology of MDA-MB-231 cancer cells was determined. In the literature, ethanol, water and DMSO extracts of propolis have been used at µg/mL concentrations for anti-cancer research [10,15]. Due to the toxic effects of these solvents in cancer cells, researchers avoid using propolis extracts at high concentration. In this study, DMEM extract of propolis was used at a concentration of 2.5 mg/mL to determine its effect on percentage cell viability, relative cell number of the tested cancer cells, when compared to the control untreated MDA-MB-231 cells. Studies using HPLC analysis have shown that water, ethanol and DMSO extracts of TP contained caffeic acid, chrysin, caffeoyl quinic acid, quercetin, pinocembrin, pinostrobin, isalpinin, pinobanksin and cinnamic acid derivatives, amongst other compounds [23-25].

It would be very illuminating to determine the chemical compositions of the DMEM extract of TP used in the present study, so as to provide additional support for the results obtained. The results revealed that the DMEM extract of TP at a concentration of 2.5 mg/mL, decreased relative cell number of the tested cancer cells, when compared to the control untreated MDA-MB-231 cells, although the effect was not significant. However, this concentration did not elicit any cytotoxic effects on the tested MDA-MB-231 cells. Moreover, process length, field diameter and cell body diameter of the treated cells decreased only in monopolar cell type, and increased in bipolar and multipolar cell types, but the changes were insignificant. Process thickness of the treated cells increased in all cell types, albeit insignificantly. Thus, the DMEM extract of TP can be employed at different concentrations for antiproliferative, cytotoxic and morphological studies on cancer cells.

## DISCUSSION

The current study is the first investigation in which the influence of DMEM extract of TP on the morphology of MDA-MB-231 cancer cells was determined. In the literature, ethanol, water and DMSO extracts of propolis have been used at µg/mL concentrations for anti-cancer research [10,15]. Due to the toxic effects of these solvents in cancer cells, researchers avoid using propolis extracts at high concentration. In this study, DMEM extract of propolis was used at a concentration of 2.5 mg/mL to determine its effect on percentage cell viability, relative cell number of the tested cancer cells, when compared to the control untreated MDA-MB-231 cells. Studies using HPLC analysis have shown that water, ethanol and DMSO extracts of TP contained caffeic acid, chrysin, caffeoyl quinic acid, quercetin, pinocembrin, pinostrobin, isalpinin, pinobanksin and cinnamic acid derivatives, amongst other compounds [23-25].

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## CONCLUSION

This study has revealed that the DMEM extract of TP at a concentration of 2.5 mg/mL results in insignificant decreases in relative cell number of MDA MB 231 cells without any significant cytotoxic and morphological effects. Further investigations are required to illustrate the effects of other concentrations and extracts of propolis on proliferation, cytotoxicity and morphology in MDA-MB-231 cancer cell lines.

## DECLARATIONS

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### Conflict of interest

No conflict of interest is associated with this work.

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## REFERENCES
