

Original Research Article

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

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Abstract

Purpose: To evaluate the influence of DMEM extract of Turkish propolis (TP) on the morphology of metastatic MDA-MB-231 cells.

Methods: The cells were incubated with DMEM extract of TP (collected from Trabzon in Turkey) at a dose of 2.5 mg/mL for 72 h. The effect of DMEM extract on proliferation and cytotoxicity of the cells was determined using 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) and trypan blue exclusion assay. MDA-MB-231 cells incubated with or without extracts were randomly photographed with a camera-coupled inverted microscope. Treated and control MDA-MB-231 cells were classified as monopolar, bipolar or multipolar, and their dimensions measured with an electronic caliper.

Results: Although the extract reduced the proliferation of the cells, the effect was not statistically significant ($p < 0.05$). Moreover, no cytotoxic effect was observed. Field diameters, process length and cell body diameters of the treated cells were increased by DMEM extract treatment in bipolar and multipolar cell types, but these parameters were decreased in monopolar cell type, although insignificantly ($p < 0.05$). In addition, the process thickness of treated MDA-MB-231 cells increased insignificantly in all cell types ($p < 0.05$).

Conclusion: These findings indicate that DMEM extract of TP at a dose of 2.5 mg/mL morphologically suppresses monopolar MDA-MB-231 cells. Future studies would examine the morphological effects of different concentrations of the propolis extract in anti-proliferation, cytotoxicity and morphological investigations in MDA-MB-231 cells.

Keywords: Turkey propolis, MDA-MB-231 cells, Proliferation, Morphology, Cytotoxicity

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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,

diarrhea, herpes and other infections; as well as breast cancer and other cancers [6-8].

Propolis has many biological activities such as anti-fungal, antibacterial, anti-viral, antiseptic, anti-inflammatory, antioxidant, immunosuppressive, immunomodulatory, anti-cancer and antiproliferative effects [9,10]. In general, propolis consists of resins (50 %), waxes (30 %), essential oils (10 %), bee pollen (5 %), organic acids and minerals (5 %), and its composition is variable depending on geographical origin, climate and period [9]. 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-angiogenic 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 1.5×10^4 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.

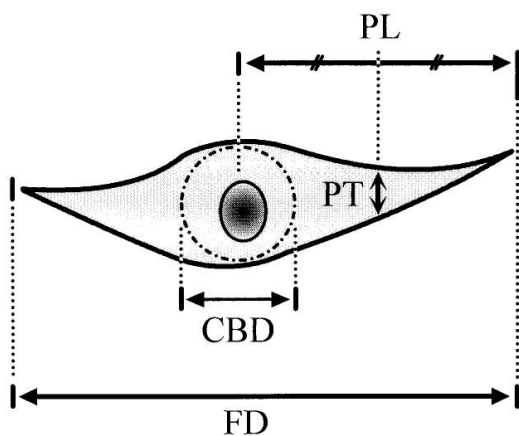


Figure 1: A schematic illustration of indices of cell morphology. PL = process length; FD = field diameter (FD); PT = process thickness (PT), and CBD = cell body diameter (CBD) [22]

Table 1: Effect of TP on relative cell number and percentage cell viability

Parameter	Propolis extract concentration (mg/mL)	
	2.5	0
Relative cell number	4.81 ± 0.24	5.50 ± 0.04
Cell viability (%)	98.74 ± 0.23	98.79 ± 0.10

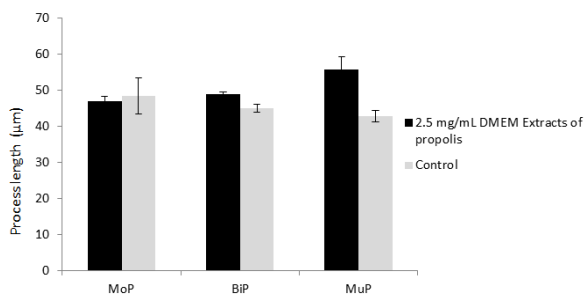


Figure 2: PL of cells treated with 2.5 mg/mL DMEM extract of TP (black columns) and control cells (grey columns). Data are presented as mean ± SEM (n = 3)

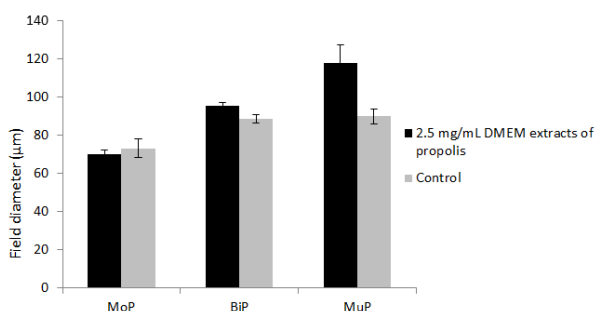


Figure 3: Field diameter of cells treated with 2.5 mg/mL DMEM extract of TP (black columns) and untreated control cells (grey columns). Data are presented as mean ± SEM (n = 3)

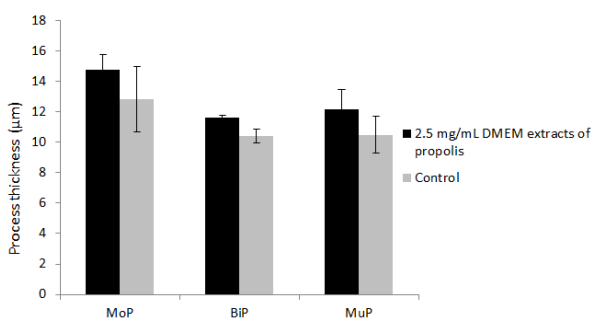


Figure 4: Process thickness of cells treated with 2.5 mg/mL DMEM extract of TP (black columns) and control cells that were not treated with TP extract (grey columns). Data are presented as mean ± SEM (n = 3)

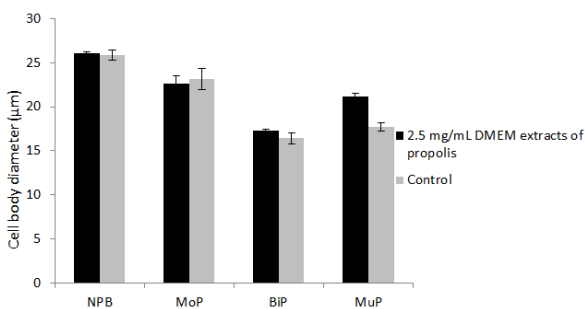


Figure 5: Cell body diameter of cells treated with 2.5 mg/mL DMEM extract of TP (black columns) and control cells that were not treated with TP extract (grey columns). Data are presented as mean ± SEM (n = 3)

Table 2: Process parameters for cells treated with 2.5 mg/mL DMEM extract of TP and those for control (untreated cells)

Parameter	Cell type	Propolis extract concentration (mg/mL)	
		2.5	0
Process length (µm)	MoP	46.95 ± 1.33	48.40 ± 5.08
	BiP	48.74 ± 0.78	44.97 ± 1.10
	MuP	55.72 ± 3.62	42.78 ± 1.53
Process thickness(µm)	MoP	14.75 ± 1.05	12.82 ± 2.15
	BiP	11.63 ± 0.16	10.42 ± 0.43
	MuP	12.15 ± 1.33	10.49 ± 1.21
Field diameter(µm)	MoP	70.35 ± 2.10	73.29 ± 4.92
	BiP	95.70 ± 1.58	88.90 ± 2.21
	MuP	117.90 ± 9.60	89.95 ± 3.97
Cell body diameter(µm)	NPB	26.09 ± 0.16	25.87 ± 0.59
	MoP	22.57 ± 0.94	23.19 ± 1.20
	BiP	17.29 ± 0.13	16.41 ± 0.58
	MuP	21.11 ± 0.43	17.71 ± 0.49

Values are mean ± SEM (n = 3)

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 and the morphology of MDA-MB-231 cells, relative to 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, pinostropin, 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.

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.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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