

Original Research Article

Evaluation of cytotoxic and wound healing effect of DMEM extracts of Turkish propolis in MDA-MB-231 cell lines

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Abstract

Purpose: To investigate the effect of Dulbecco's Modified Eagle Medium (DMEM) extract of Turkish propolis on proliferation, cytotoxicity and lateral motility in MDA-MB-231 cells.

Methods: The antiproliferative activity of DMEM extracts of propolis was determined colorimetrically in MDA-MB-231 cells using 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay. Cell toxicity and wound healing effects of the propolis extracts were determined with trypan blue exclusion assay and wound-healing assay, respectively.

Results: The cell number of MDA-MB-231 cells were decreased by the extracts at all concentrations for 72 h. The highest antiproliferative activity of the extract was demonstrated at 10 mg/mL for 24 - 72 h. Moreover, 5 and 0.31 mg/mL of the propolis extract showed significant antiproliferative activity at 72 h of incubation. The extract showed cytotoxic effect to MDA-MB-231 cells at 10 mg/mL. The extract (at a dose of 2.5 mg/mL) during 24 - 72 h did not produce any effect on lateral motility in MDA-MB-231 cells in the wound healing assay.

Conclusion: These results indicate that the DMEM extract of propolis exerts antiproliferative and cytotoxic effects on MDA-MB-231 cells at different concentrations.

Keywords: Propolis, Proliferation, Cytotoxicity, Motility, Breast cancer

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INTRODUCTION

Each year, more than 14.1 million people are diagnosed with cancer and most of them live in low and middle income countries [1]. The most common cancer type among woman in the United States between 1975 and 2014 was breast cancer [2]. Recently, researchers have investigated chemotherapeutic or complementary roles of natural compounds in the treatment of cancer [3,4]. Propolis is collected from gummy

plants and trees by bees (*Apis Mellifera*) for repairing splits in their hive, and defending the hive from invaders and diseases. Propolis has been applied in folk medicine and apitherapy for centuries due to its pharmaceutical and biological properties such as immunomodulatory, wound healing, antitumoral, antimicrobial and antioxidant activities [5,6].

The major constituents of propolis are flavonoids, phenolic acids and their esters (about 50 %);

fatty acids and waxes (about 30 %); and essential and aromatic oils, pollen and other organic substances and minerals making up 20 %, with composition influenced by factors such as geographic area, climate and type of plants and trees that bees tap from [7,8]. Turkish propolis is rich from in flavonoids and phenolics such as naringenin, quercetin, pinocembrin, caffeic acid, apigenin, caffeic acid phenyl ester, pinobanksin, galangin, chrysin and cinnamic acids [9,10]. Studies have shown that many biological activities of propolis including antitumor activity may be related to its flavonoid and phenolic acid compositions [11-14]. Ethanol, methanol, water, dimethyl sulfoxide (DMSO), polyethylene glycol (PEG), oil, hexane and ethyl acetate have been used as solvents for extraction of propolis [14-16]. The present study is the first to use DMEM as a solvent for extracting propolis.

The aim of this study was to investigate the antiproliferative, cytotoxic and lateral motility effects of DMEM extracts of Turkish propolis on MDA-MB-231 cells.

EXPERIMENTAL

Chemicals

L-glutamine-containing DMEM, DMEM without glutamine, glucose and phenol red, FBS, penicillin–streptomycin, MTT, trypan blue, DMSO, glycine, trypsin, ethylenediamine-tetraacetic acid (EDTA) and NaCl were supplied by Sigma (United Kingdom).

Preparation of DMEM extract of Turkish propolis

Propolis samples were collected from Trabzon in Turkey (Fanus Food Company, Trabzon). Five gram of the sample was ground and kept at -20 °C. The ground propolis sample was dissolved in 20 mL of DMEM without glucose, glutamine or phenol red, by continuous shaking at 150 rpm in a 60 °C water bath for 24 h. The extract was centrifuged for 10 min at 4000 rpm, and then subjected to microfiltration and sterilisation to obtain a stock solution of concentration 250 mg/mL which was kept away from light at 4 °C. Various concentrations of working solutions (10, 5, 2.5, 1.25, 0.63, 0.31 and 0.16 mg/mL) were prepared by diluting the stock of 250 mg/mL with DMEM.

Cell culture

MDA-MB-231 breast cancer cells were purchased from American Type Culture

Collection (ATCC, USA). The cells were maintained in DMEM containing 4 mM L-glutamine and 5 % FBS, and incubated at 37 °C in a 100 % humidity atmosphere with 5 % CO₂. The cancer cells were passaged every 3 - 4 days with a solution containing trypsin (0.25 %) and EDTA (0.02 %) [17].

Cell viability assay

Cell viability of MD-MB-231 cells was assayed using trypan blue exclusion following incubation for 24, 48 and 72 h with normal growth medium DMEM, and propolis extract at doses of 1.25, 2.5, 5.0, and 10.0 mg/mL. The number of dead and live cells was assessed microscopically from 30 fields of view randomly selected for that purpose [18]. The results were obtained from 3 separate experiments.

MTT cytotoxicity assay

The MDA-MB-231 cells were seeded overnight into 24-well plates at a density of 1.5×10^4 cells/well. Proliferation of MDA-MB-231 cells were determined colorimetrically using MTT assay [19].

Wound healing assay

Lateral motility was determined with wound healing assay in the MDA-MB-231 cells with and without treatment with DMEM extract of propolis. A marker pen was used to draw parallel lines on the reverse side of empty 35 mm petri dishes. The cells were seeded at 5×10^4 cells in 35 mm petri dishes and subjected to incubation for 24 h at 37 °C. The cultured cells were scratched with a 200- μ l tip and washed 4 times with culture media DMEM. Then, they were treated with and without 2.5 mg/mL DMEM extract of propolis for 24, 48 and 72 h. The space from scratch treatment between control and treated culture cells were quantified by using inverted microscope appearance (ID 03 Carl Zeiss Ltd, Welwyn Garden City, UK). Wound healing assays were repeated four times [20].

Statistical analysis

Cell viability and MTT assays were repeated thrice, while wound healing assay was repeated four separate times to ensure accurate results. All results are expressed as mean \pm standard error of the mean (SEM). Student's *t*-test was used for comparing the effect of DMEM extracts of propolis on MDA-MB-231 cells and control (SPSS 20.0, IBM, Armonk, NY, United States of America). Values of $p < 0.05$ were taken as indicative of statistical significance of differences.

RESULTS

DMEM extract of Turkish propolis showed anti proliferative effect relative to control cells in 72 h at all concentration of 0.16 up to 10 mg/mL. The extract significantly decreased cell number of MDA-MB-231 cells at doses 10, 5, and 0.31 mg/mL, as shown in Figure 1. In addition, the results of trypan blue assay were verified with MTT results. All data are shown at Table 1. The DMEM extract of propolis at a dose of 10 mg/mL showed cytotoxic effect on MDA MB 231 cells. However, at a dose of 5 mg/mL, the DMEM extract of propolis did not show cytotoxic effect, although it produced some morphological changes in MDA MB 231 cells (results not presented). Arising from the results of the MTT and cell viability assays, 2.5 mg/mL.

DMEM extract of propolis was chosen for investigating the wound healing effects of extracts on MDA-MB-231 cells. It was revealed that 2.5 mg/mL DMEM extracts of propolis did not significantly change wound healing effect in MDA-MB-231 cells, when compared to cells in the control group. The results of wound healing effects of DMEM extracts of propolis are shown in Table 2.

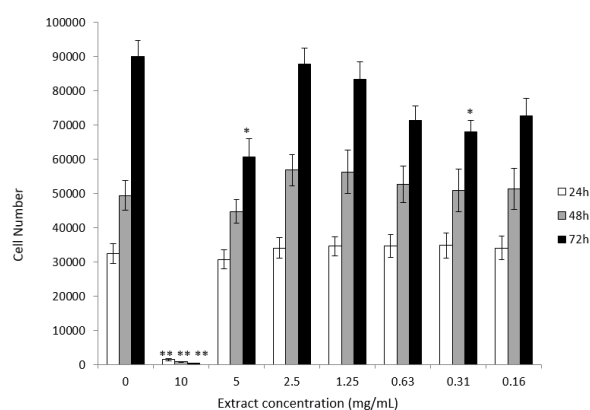


Figure 1: Mean cell number of MDA-MB-231 cells incubated with DMEM extracts of propolis and control MDA-MB-231 cells for 24, 48 and 72 h. Data are presented as mean \pm SEM, $n = 3$; * $p < 0.05$, ** $p < 0.01$

Table 1: Cell viability of MDA-MB-231 cells incubated with different concentrations of DMEM extract of propolis (10, 5, 2.5, 1.25 mg/mL) for 24, 48 and 72 h

Time (h)	Concentration of DMEM extract of propolis (mg/mL)				
	0	1.25	2.5	5	10
24	99.20 \pm 0.15	99.59 \pm 0.20	99.66 \pm 0.09	99.96 \pm 0.02	98.69 \pm 0.76
48	99.00 \pm 0.36	99.57 \pm 0.11	99.63 \pm 0.15	99.15 \pm 0.38	82.75 \pm 12.91
72	98.29 \pm 0.44	98.38 \pm 0.46	99.03 \pm 0.41	97.86 \pm 0.57	70.04 \pm 13.84

Values are mean \pm SEM ($n = 3$)

Table 2: Motility index of control MDA-MB-231 cells incubated with 2.5 mg/mL of DMEM extract of propolis

Time (h)	MI of Control MDA-MB-231 cells (\pm SEM)	MI of treated MDA-MB-231 cells (\pm SEM)
24	0.34 \pm 0.03	0.41 \pm 0.02
48	0.57 \pm 0.04	0.68 \pm 0.03
72	0.82 \pm 0.05	0.90 \pm 0.03

Values are mean \pm SEM ($n = 4$)

DISCUSSION

This study is the first to use DMEM as a solvent for extraction of propolis. In the literature, ethanol is usually used for preparation of extracts of propolis. Extracts of propolis made with DMSO were used at μ g/mL concentration because of the toxic effect of the solvent [13,21-23]. Higher concentrations of DMEM propolis extract or extracts prepared with other cell culture media can be used in cell culture experiments. In previous studies, it was determined that DMSO extracts of Turkish propolis contained naringenin, galangin, chrysin, quercetin, kaempferol and cinnamic acid derivatives, while water extracts of Turkish propolis contained caffeic and caffeoyl quinic acids, as revealed by HPLC analysis [24]. Flavonoid and phenolic compounds of DMEM extracts of propolis should be investigated for supporting data. In many studies, the cytotoxic effects of propolis extract were seen at microgram levels.

The DMEM extract of propolis showed cytotoxic effects at milligram levels. Thus, 10 mg/mL of the extract of propolis showed strongly antiproliferative and cytotoxic effects in MDA-MB-231 cells. However, 2.5 mg/mL DMEM extracts of propolis did not show delayed effect on invasion in MDA-MB-231 cells. The DMEM extract of propolis may be used for further investigations and may be an alternative extract for antiproliferative, cytotoxic and antimetastatic investigations with cancer cells.

CONCLUSION

The findings of the present study show that DMEM extracts of Turkish propolis have antiproliferative and cytotoxic effects, but at a dose of 2.5 mg/mL, it does not exert a wound healing effect on MDA MB 231 cells. Thus, DMEM extract of propolis may be a suitable alternative apitherapy extract for cancer research.

DECLARATIONS

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Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors 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 them.

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