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Influence of polyphenol extract from evening primrose *(Oenothera paradoxa)* seeds on human prostate and breast cancer cell lines*

Wpływ ekstraktu polifenolowego z nasion wiesiołka dziwnego *(Oenothera paradoxa)* na komórki nowotworowe prostaty i piersi

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

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Summary

There is growing interest in plant polyphenols which exhibit pleiotropic biological activities, including anti-inflammatory, antioxidant, and anticancer effects. The objective of our study was to evaluate the influence of an evening primrose extract (EPE) from defatted seeds on viability and invasiveness of three human cell lines: PNT1A (normal prostate cells), DU145 (prostate cancer cells) and MDA--MB-231 (breast cancer cells). The results revealed that after 72 h of incubation the tested extract reduced the viability of DU 145 and MDA-MB-231 with IC50 equal to 14.5 μg/mL for both cell lines. In contrast, EPE did not inhibit the viability of normal prostate cells. Furthermore, EPE reduced PNT1A and MDA-MB-231 cell invasiveness; at the concentration of 21.75 μg/mL the suppression of invasion reached 92% and 47%, respectively (versus control). Additionally, zymographic analysis revealed that after 48 h of incubation EPE inhibited metalloproteinase-2 (MMP-2) and metalloproteinase-9 (MMP-9) activities in a dose-dependent manner. For PNT1A the activities of MMP-2 and MMP-9 decreased 4- and 2-fold, respectively, at EPE concentration of 29 μg/mL. In the case of MDA-MB-231 and DU 145 the decrease in MMP-9 activity at EPE concentration of 29 μg/mL was 5.5-fold and almost 1.9-fold, respectively. In conclusion, this study suggests that EPE may exhibit antimigratory, anti-invasive and antimetastatic potential towards prostate and breast cancer cell lines.

Keywords: evening primrose • polyphenols • matrix metalloproteinase • invasiveness • cancer

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Introduction

The increasing interest in biological activities of polyphenols observed in recent years is caused by, among others, an increase in the incidence of diseases associated with life style (cardiovascular diseases, diabetes, cancer and neurodegenerative diseases) as well as by a need to search for effective and safe methods of prophylaxis and/or therapy. Results of epidemiological studies indicate that many of the above-mentioned diseases could be prevented by enriching one's diet in fruits, vegetables and cereals [27,39]. Natural phenolic compounds exhibit antioxidative, anti-inflammatory, antiallergic and anticancer activities [1,27,30]. Health beneficial effects of polyphenols result not only from counteracting oxidative stress underlying numerous diseases [24,25] but also from interactions with signal transduction pathways [6,14] as well as from inhibition of certain enzymatic activities [22,37]. Polyphenols with documented chemopreventive activities include epigallocatechin gallate (EGCG), curcumin, resveratrol and genistein [6,7,9,37].

Some polyphenols also act as inhibitors of matrix metalloproteinases (MMPs), which are proteolytic enzymes digesting extracellular matrix (ECM) proteins [3,8]. MMPs play a key role in many physiological processes such as embryogenesis and wound healing; however, the same enzymes are involved in pathogenesis of many diseases, including cancer, atherosclerosis and diabetes [4,23,29]. Among matrix metalloproteinases, MMP-2 and MMP-9 are particularly active in ECM degradation; they participate in primary tumor vascularization and in the formation of distant metastases [13]. The interest in the correlation between MMP activities and tumor development is therefore warranted. At present, this group of enzymes is considered to be an important target for new antimetastatic drugs that would inhibit their activities.

The aim of our study was to assess selected biological activities of a crude polyphenol extract from defatted seeds of evening primrose (*Oenothera paradoxa* Hudziok); this medical plant has not been widely investigated in that respect so far. Evening primrose belongs to the *Onagraceae* family that originated in North America and is cultivated in Europe, North America and New Zealand for its seeds used as a source of unsaturated fatty acids, particularly γ-linolenic acid (GLA) [31]. There are a number of reports on the health beneficial effects of evening primrose oil; in contrast, papers regarding the activities of polyphenols extracted from this plant are still scarce [2,15,20,22,32]. In the present work we assessed the influence of EPE on viability and invasiveness of three human cell lines – normal prostate cells (PNT1A), prostate cancer cells (DU 145) and breast cancer cells (MDA-MB-231) – as well as on MMP-2 and MMP-9 activities in the above-mentioned cells.

Materials and methods

Plant materials

Evening primrose (*Oenothera paradoxa* Hudziok) defatted seeds were obtained from Agropharm S.A./Adamed Group pharmaceutical company (Tuszyn, Poland).

Chemicals

Chlorogenic acid, gallic acid, ellagic acid, (+)-catechin, (–)-epicatechin, quercetin 3-rhamnoside, and methyl gallate were purchased from Sigma-Aldrich (St. Louis, MO, USA). Cryptochlorogenic acid, dimer B1, dimer B2, and trimer C1 were purchased from PhytoLab GmbH & Co. KG (Vestenbergsgreuth, Germany). HPLC grade acetonitrile was purchased from J.T. Baker (Griesheim, Germany). All other chemicals were reagent grade products purchased from POCH S.A. (Gliwice, Poland).

Polyphenol extract preparation

Evening primrose dry polyphenol extract was obtained according to the procedure described by Gorlach et al. [15]. The resulting extract is referred to as EPE in this work. The dry EPE was stored at -20°C prior to further analyses.

Characterization of the polyphenol extract

The extract was characterized in terms of total polyphenol content by the Folin-Ciocalteu procedure, expressed as (+)catechin equivalents [5], total flavanol content by the vanillin procedure, expressed as (+)catechin equivalents [35], and total proanthocyanidin content after acid hydrolysis in a butanol environment, expressed as cyanidin [34]. Ellagitannins and gallotannins (after acid hydrolysis in a methanol environment, 20 h, 85°C) were determined by an analytical reversed-phase HPLC system with the use of a Eurospher-100 C18 column (250 mm x 4.6 mm, 5 µm) (Knauer, Berlin, Germany). A binary mobile phase and a gradient program were the same as described below. Ellagitannin content determined at 254 nm is expressed as ellagic acid and gallotannin content at 280 nm is expressed as methyl gallate [16].

Phenolics determination by HPLC-DAD

The phenolic profile was determined using an analytical reversed-phase HPLC system (Waters, Milford, MA) with a 2707 autosampler and a 1525 binary HPLC pump coupled to a 996 photodiode array detector (2998), controlled by Waters Breeze 2 software (Waters). A SYMMETRY C18 column (250 mm x 4.6 mm, 5 µm) (Waters) was used. According to Dyrby et al. [10], the binary mobile phase consisted of water and formic acid in the ratio of 90:10 (v/v), respectively (solvent A); and water, acetonitrile and formic acid in the ratio of 49:50:10 (v/v/v), respectively (solvent B). The phenolic separation was performed using the following gradient program with a

flow rate of 1 ml·min^{-1}: 0 min, 88% A + 12% B; 26 min, 70% A + 30% B; 40-43 min, 0% A + 100% B; 48-50 min, 88% A + 12% B. Detection was performed by scanning from 200 to 550 nm. Peak identification was carried out by comparison of retention times and diode array spectral characteristics with the standards.

Cells and culture conditions

Normal human prostate PNT1A cells, human prostate cancer DU 145 cell line, and human breast cancer MDA-MB-231 cell line were purchased from Health Protection Agency Culture Collections (London, UK). DU 145 and MDA-MB-231 cells were cultured in MegaCell Dulbecco's Modified Eagle's Medium (MC DMEM) supplemented with 3% fetal bovine serum (FBS), 4 mmol/L L-glutamine, 50 U/mL penicillin, 50 µg/mL streptomycin, 100 µg/mL neomycin, 1.25 µg/mL amphotericin B, 1 mmol/L sodium pyruvate, and 1% MEM non-essential amino acids. PNT1A cells were cultured in RPMI-1640 medium supplemented with 10% FBS, 2 mmol/L L-glutamine, 50 U/mL penicillin, 50 µg/mL streptomycin, 1.25 µg/mL amphotericin B. Cells were grown in a humidified atmosphere of 5% CO_2 at 37ºC. The cells were seeded in such quantity that the confluence at the end of the experiment did not exceed 80% in control wells. In the present work EPE was tested within the concentration range from 7.25 µg/mL to 29 µg/mL. For all bioassays EPE was dissolved in 70% ethanol solution in deionized water and then diluted to the final concentration with a serum-free medium. The final concentration of ethanol was lower than 0.01% (v/v). Controls for the EPE treatment were incubated with ethanol.

Viability assay

Cell viability was assessed after 24, 48, and 72 h incubation with or without EPE (0 µg/mL – 29 µg/mL) by crystal violet staining. Crystal violet staining is a colorimetric method which dyes cellular nuclei. The staining was performed according to Henriksson et al. [17] with slight modifications. Briefly, the cells were harvested, suspended in the growth medium mentioned above, and seeded on 96-well plates. PNT1A, DU145, and MDA-MB-231 cells were seeded at the densities of 2.5 x 10^3, 2.0 x 10^3, and 3.0 x 10^3 per well, respectively. After 24 h, the cells were exposed to the EPE for 24, 48 and 72 h. After culture, the medium was removed and the cells were fixed *in situ* with 4% formaldehyde solution in PBS for 30 min at room temperature. Then the cells were washed twice with PBS (pH 7.4) and stained with 0.5% crystal violet dissolved in 25% aqueous solution of methanol for 5 min at room temperature. Unbound dye was washed out with deionized water and the cells allowed to air dry. The dye was solubilized in 33% aqueous solution of acetic acid while shaking for 30 min at room temperature. Optical density (OD) was measured with a microplate reader (iMark™, BioRad Laboratories, Hercules, CA, USA) at the wavelength of 595 nm.

Invasion assay

Invasion studies were conducted using the Matrigel BM matrix assay developed for measurement of tumor cell invasiveness, as described previously [26]. We used BioCoat Matrigel invasion chambers (24-well cell culture inserts containing an 8.0-µm PET membrane with a uniform layer of Matrigel [Becton Dickinson, Bedford, MA]). The lower chamber contained medium with 10% FBS as a chemoattractant. Cells were resuspended in serum-free medium with or without EPE (0 µg/mL – 21.75 µg/mL), and plated onto the upper chamber (cell density of 5 x 10^4/mL) according to the manufacturer's recommendations. The chambers were incubated for 48 h at 37°C in a 5% CO_2 atmosphere. After incubation the non-migrated cells in the upper chamber were gently scraped away and adherent cells present on the lower surface of the insert were stained with crystal violet, photographed, and counted using NIH ImageJ analysis software.

Gelatin zymography

In order to determine the influence of EPE on MMP-9 activity, the enzyme expression was stimulated with tumor necrosis factor α (TNF-α) and/or 12-O-tetradecanoylphorbol-13-acetate (TPA) [21]. For zymographic analysis we chose the lowest concentrations of TNF-α and/or TPA which were able to induce MMP-9 activity in each cell line: 5 ng/mL TPA (PNT1A), 10 ng/mL TNF-α + 10 ng/mL TPA (DU 145), and 25 ng/mL TNF-α + 25 ng/mL TPA (MDA-MB-231). The cells were harvested, suspended in the growth medium mentioned above, and seeded on 96-well plates. PNT1A, DU145, and MDA-MB-231 cells were seeded at the densities of 3.5 x 10^3, 5.0 x 10^3, and 3.0 x 10^3 per well, respectively. After 24 h, the cells were washed twice with PBS. Next, the cells were incubated in serum-free media in the presence of the above-mentioned stimulants and with or without EPE (0 µg/mL – 29 µg/mL) for 48 h. Gelatin zymography of cell culture media samples was performed as described previously [18]. Briefly, the same volumes of media (20 µl) were dissolved in electrophoresis sample buffer containing sodium dodecyl sulfate (SDS) and subjected to electrophoresis in a 10% polyacrylamide gel embedded with gelatin (1.5 mg/mL) in the absence of β-mercaptoethanol. After electrophoresis type IV collagenases (MMP-2 and MMP-9) were renatured by incubation with 2.5% Triton X-100, and the enzyme reaction was allowed to proceed at 37°C for 21 h. Thereafter, the gels were stained for 1.5 h with 0.0125% Amido Black in 7% acetic acid and 20% ethanol. Type IV collagenases (MMP-2 and MMP-9) were visualized without destaining as transparent bands against the dark blue background of Amido Black-stained slab gels. The intensity of bands was quantified by densitometric analysis using the Gel-Doc™ EQ system with Quantity One software (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

Statistical analysis

Data are presented as mean ± SD or SEM, as indicated in figure legends. The number of independent experiments is given in figure legends. Statistical significance of differences between means was determined by nonparametric analysis (Kruskal-Wallis one-way ANOVA followed by Bonferroni test) with the use of Analyze-it software, v. 2.21 (Analyze-it Software, Ltd., Leeds, UK); p values of < 0.05 were considered to be statistically significant.

Results

Characterization of the composition of EPE

The content of total polyphenols and several groups of those compounds in EPE were determined by HPLC (Table 1). A representative chromatogram for the analysis of EPE components is depicted in Figure 1. The total polyphenol content was 578.15 ± 15.76 mg/g, including 190.02 ± 5.32 mg/g (28.9%) of flavanol mono-, oligo-, and polymers. (+)-Catechin and (-)-epicatechin (flavanols monomers) constituted 2.0% and 1.96% of total polyphenols, respectively. Besides, EPE contained gallic acid in its free form (0.9%) and in the form of esters, namely, gallotannins (15.4%). In the present work EPE was tested within the concentration range from 7.25 to 29 μg/mL.

Influence of EPE on viability of PNT1A, DU 145, and MDA-MB-231 cells

As depicted in Fig. 2A, EPE did not have a negative influence on human normal prostate PNT1A cell viability. EPE stimulated PNT1A cell viability at the whole concentration range chosen for the study. The stimulation levels were comparable for all the incubation times; after 72 h they were 48%, 60%, 58%, and 56% at EPE concentrations of 7.25 μg/mL, 14.5 μg/mL, 21.75 μg/mL, and 29 μg/mL, respectively (versus control). In the case of both human cancer cell lines (DU145 and MDA-MB-231) EPE decreased their viability after 72 h incubation (IC_{50} was 14.5 μg/mL for both cancer cell lines). At EPE concentration of 29 μg/mL the decrease in cell viability reached even 82% and 88% for DU 145 and MDA-MB-231, respectively (versus control). For normal PNT1A cells IC_{50} was not reached in the whole concentration range (Figure 2B).

Table 1. Characteristics of EPE

Spectrophotometric analysis [mg/g]	
Total polyphenols a)	578.15 ± 13.63
Total flavanols b)	190.02 ± 5.32
Total proanthocyanidins c)	142.86 ± 4.05
HPLC analysis [mg/g]	
Total flavanols and HBAd)	228.05 ± 4.65
(+)catechin (monomer)	11.77 ± 1.24
(-)epicatechin (monomer)	11.36 ± 0.87
Procyanidin B1 (dimer)	7.34 ± 0.36
Procyanidin B2 (dimer)	19.47 ± 0.95
Procyanidin C1 (trimer)	7.84 ± 1.24
Ellagitannins e)	4.12 ± 0.19
Gallotannins f)	89.08 ± 8.13
Free ellagic acid	2.65 ± 0.23
Free gallic acid	5.17 ± 0.11

Mean ± SD, $n^3$3. Values are expressed per gram of dry extract. a) determined by Folin-Ciocalteu reagent as gallic acid equivalents; b) determined by vanillin reagent as (+)-catechin equivalents; c) determined by acid hydrolysis as cyanidin equivalents; d) determined by HPLC at 280 nm as gallic acid equivalents; e) determined by HPLC at 254 nm by acid hydrolysis as ellagic acid; f) determined by HPLC at 280 nm by acid hydrolysis as methyl gallate; HBA – hydroxybenzoic acids.

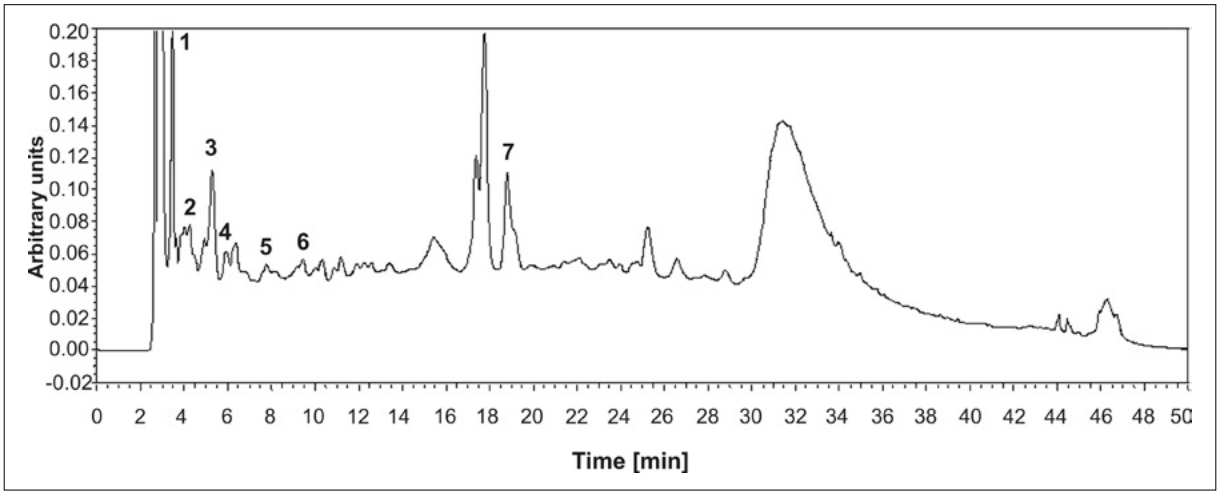
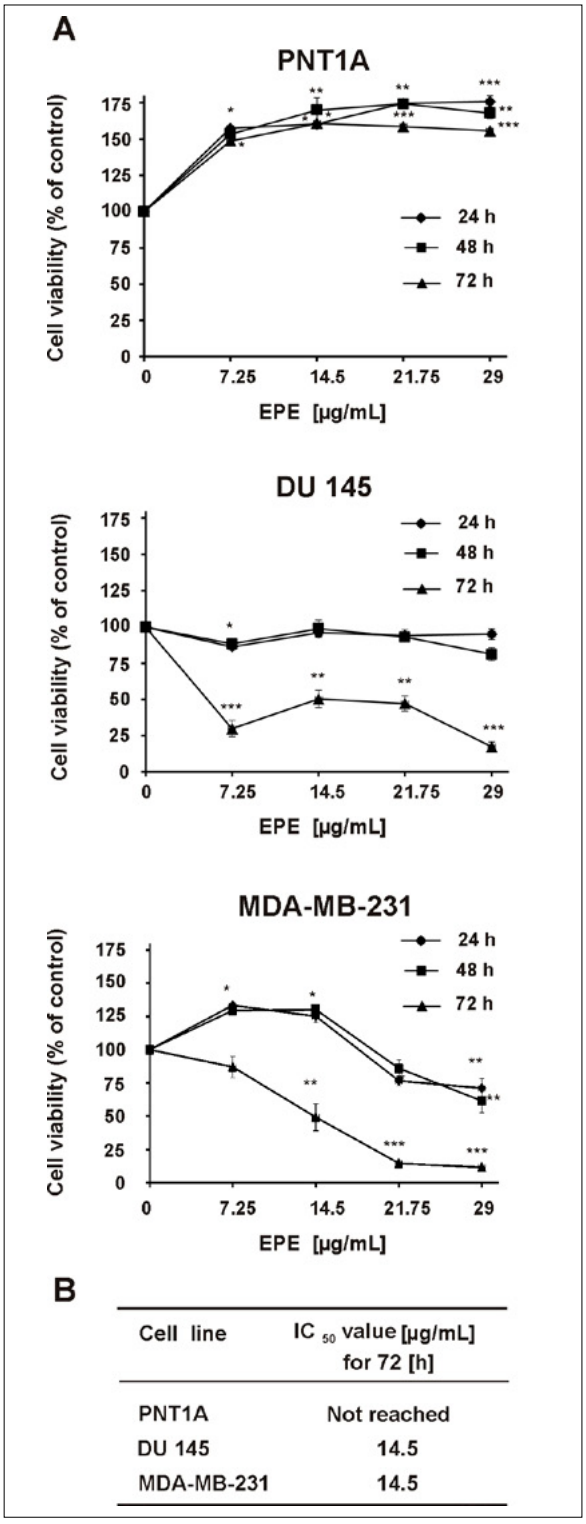


Fig. 1. HPLC phenolic profile of EPE. Detection at 280 nm. Peaks: (1) gallic acid; (2) procyanidin B1 (dimer); (3) procyanidin B2 (dimer); (4) (+)-catechin (procyanidin monomer); (5) procyanidin C1 (trimer); (6) (-)-epicatechin (procyanidin monomer); (7) ellagic acid



Cell line	IC_{50} value [µg/mL] for 72 [h]
PNT1A	Not reached
DU 145	14.5
MDA-MB-231	14.5

Fig. 2. Influence of EPE on PNT1A, DU 145, and MDA-MB-231 cell viability determined on the basis of crystal violet staining. Data are expressed as the percentage of control (untreated cells) (A). Concentrations of EPE causing a 50% inhibitory effect on cell viability when compared with untreated cells (IC_{50} value) (B). Each value represents the mean value ± SD, n = 3 independent experiments (each experiment was carried out in four replicates). Significance of differences between means: *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$ versus control

Influence of EPE on invasiveness of cancer cells

In this study we also tested whether EPE inhibits the invasion ability of cells. Invasion assay in a Matrigel-coated transwell chamber showed that EPE suppressed the invasiveness of PNT1A and MDA-MB-231 cells (Figure 3A, 3B and 3C). The suppression of PNT1A invasiveness reached 44%, 64% and 92% at EPE concentrations of 7.25 µg/mL, 14.5 µg/mL, and 21.25 µg/mL, respectively (versus control). In the case of MDA-MB-231 cells the inhibition of invasion reached 21%, 32% and 47% after 48 h of incubation with EPE at the concentrations of 7.25 µg/mL, 14.5 µg/mL and 21.25 µg/mL, respectively. EPE did not inhibit DU 145 invasiveness.

Influence of EPE on activity of type IV collagenase

As demonstrated in Figure 4A, we detected pro-MMP-9 (92 kDa) and pro-MMP-2 (72 kDa) in cultured media. EPE inhibited the activities of both MMP-2 and MMP-9 secreted by PNT1A. At EPE concentration of 7.25 µg/mL, 14.5 µg/mL, 21.25 µg/mL and 29 µg/mL MMP-9 activity decreased to 83%, 61%, 59% and 50%, whereas for MMP-2 the reduction was 63%, 56%, 42% and 25%, respectively. For DU 145 cells MMP-9 activity was reduced to 79%, 71%, 53% and 53% at EPE concentrations of 7.25 µg/mL, 14.5 µg/mL, 21.25 µg/mL and 29 µg/mL, respectively. In the case of MDA-MB-231 cells, under the influence of the tested polyphenol extract MMP-9 activity decreased to 72%, 48%, 20% and 18% at EPE concentrations of 7.25 µg/mL, 14.5 µg/mL, 21.25 µg/mL and 29 µg/mL, respectively (Figure 4B). MMP-2 activity was not detected in culture media of DU 145 and MDA-MB-23.

Discussion

Evening primrose defatted seeds (defatted seed cake) are a rich source of various polyphenols and are obtained in large amounts as a waste product of pharmaceutical and cosmetic industries [22]. We obtained a crude extract from the defatted seed cake and we determined its phenolic profile by means of HPLC (Figure 1, Table 1).

Afterwards, we investigated selected biological activities of EPE towards human normal immortalized prostate cells (PNT1A), human prostate cancer cells (DU 145) and human breast cancer cells (MDA-MB-231). Taking into account that polyphenols are antioxidants and therefore reduce tetrazolium salts to formazan [36], we did not use the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay in our experimental setup. As crystal violet staining is independent of redox reactions, we used this method instead of the MTT assay to determine the influence of EPE on the viability of the above-mentioned cell lines [33].

As shown in Figure 2A, after 72 h of incubation EPE reduced the viability of DU 145 and MDA-MB-231 cells and IC_{50} was 14.5 µg/mL for both cancer cell lines; however, the effect of EPE on cell viability was different

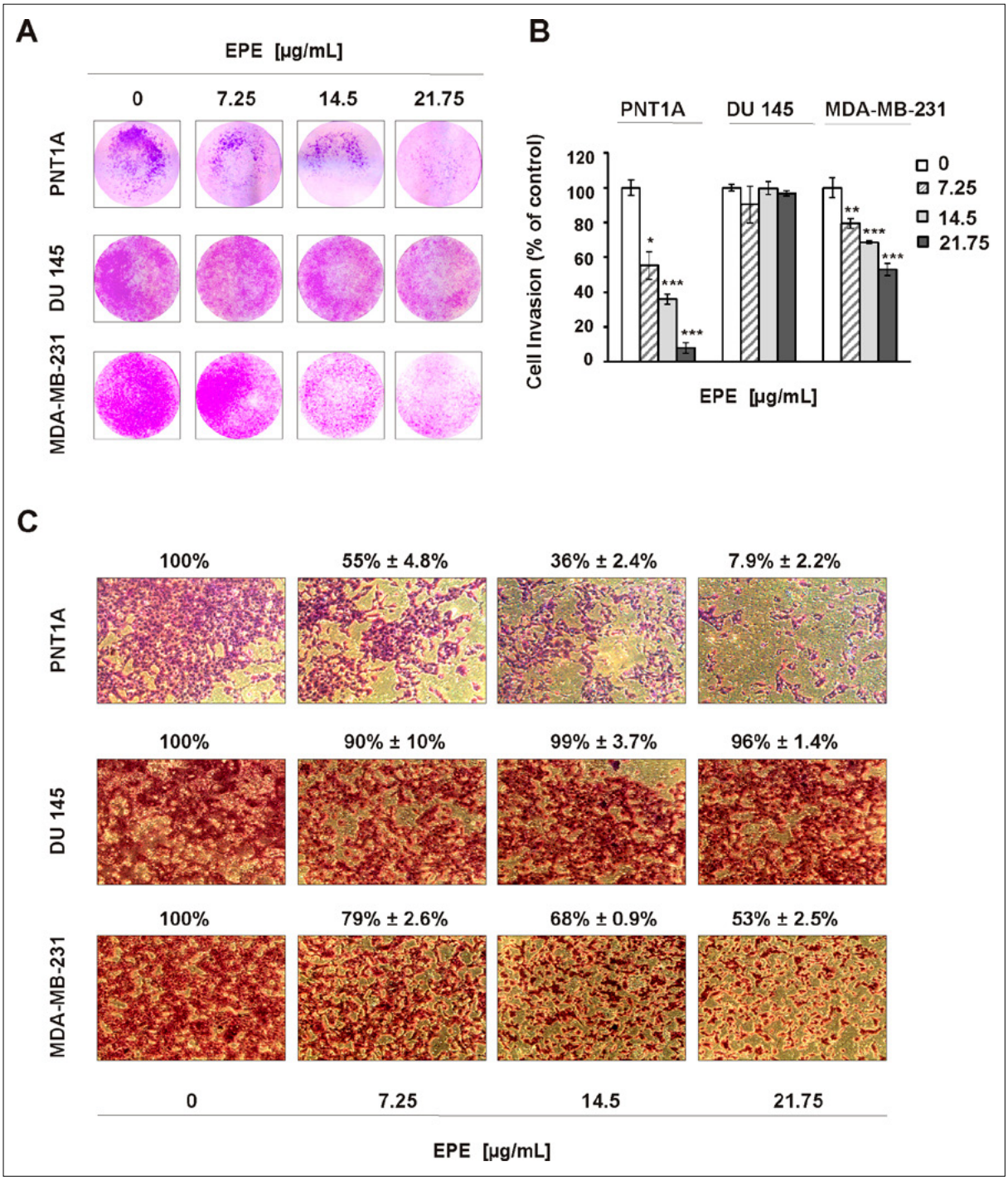


Fig. 3. Influence of EPE on invasiveness of PNT1A, DU 145, and MDA-MB-231 cells. EPE was used in the concentration range 7.25 µg/mL to 21.75 µg/mL. Invasion studies were conducted using the Matrigel BM matrix assay developed for measurement of tumor cell invasiveness. Total area of inserts with crystal violet-stained cells was photographed and counted using NIH ImageJ analysis software. (A) Exemplary images of cell growth in Matrigel after 48 h of culture. (B) Each value on the graph represents the mean value ± SD, n = 3 independent experiments (each experiment was carried out in triplicate). Significance of differences between means: *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$ versus control. (C) Images of representative fields within insert area from one typical experiment are shown. Cells were photographed after 48 h of incubation with or without EPE using an inverted Motic AE31 microscope at 200x initial magnification

in each of the cell lines (Figure 2B). Interestingly, our research demonstrated that EPE had markedly different effects on viability of normal versus cancer cells (Figure 2). EPE stimulated the viability of normal prostate PNT1A cells in the whole concentration range and for all incubation times (after 72 h of incubation with EPE at the concentration of 14.5 µg/mL the stimulation reached 60%). On the other hand, stimulation of normal prostate

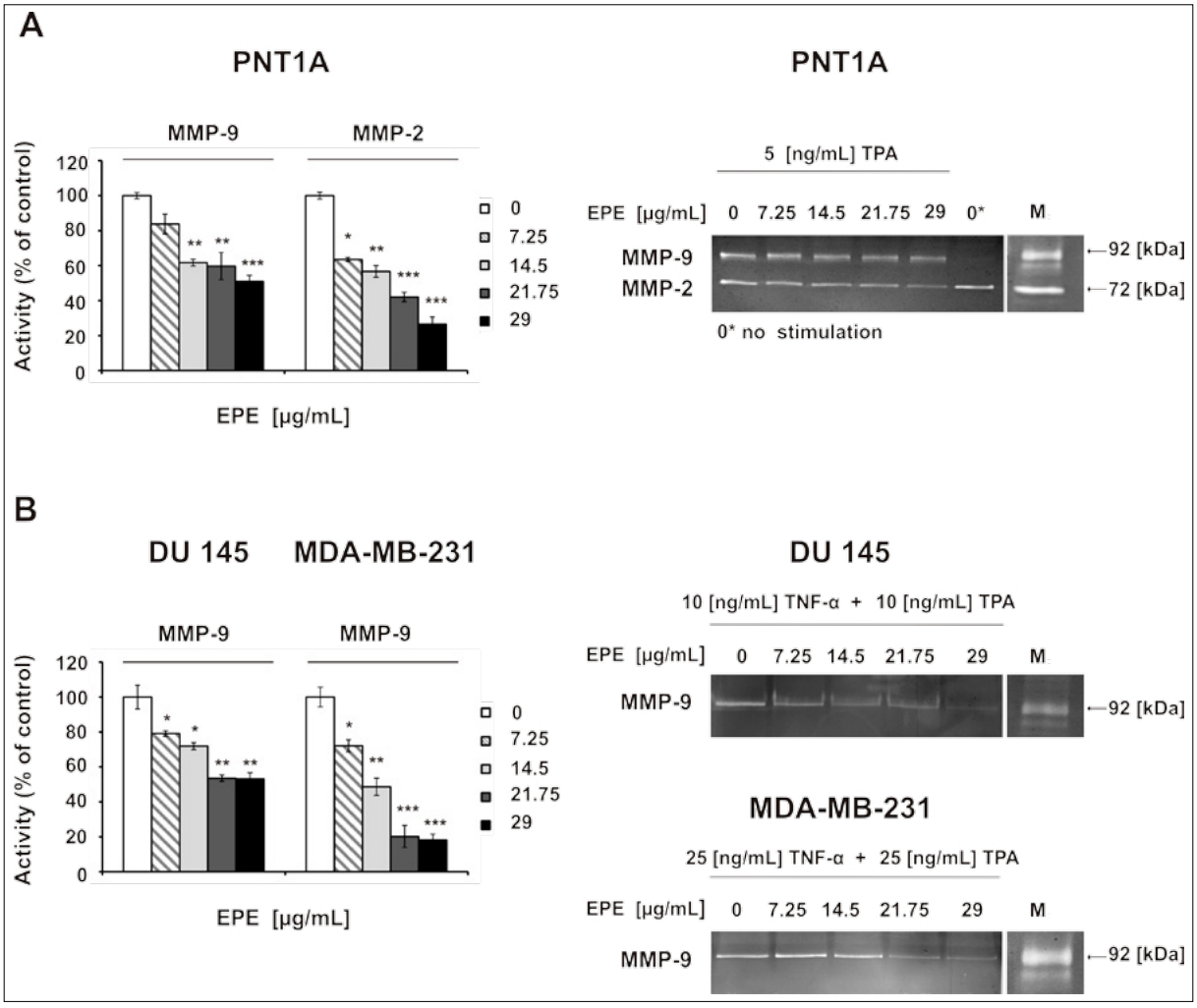


Fig. 4. Inhibition of type IV collagenases (MMP-2 and MMP-9) activities after incubation of PNT1A cells with EPE (A). Inhibition of activity of MMP-9 after incubation of DU 145 and MDA-MB-231 cell lines with EPE (B). Zymographic analysis of the media was carried out after 48 h incubation with or without EPE. Representative zymograms obtained after 48 h incubation with EPE are shown next to graphs. Each value on the graphs represents the mean value ± SEM, n ≥ 3 independent experiments. Significance of differences between means: *, $p < 0.05$ versus control. M = protein molecular weight marker

cell viability by EPE may result in undesirable changes in the form of benign prostate hyperplasia (BPH) [38].

The influence of a crude polyphenol extract from evening primrose defatted seeds (such as EPE) on the viability of PNT1A, DU 145 and MDA-MB-231 cells has not been investigated so far. However, there are reports on the effects of extracts from evening primrose defatted seeds on other cell lines [15,20]. Gorlach et al. [15] investigated the influence of the same polyphenol extract as in our studies on human colorectal adenocarcinoma Caco-2 and HT-29 cell lines and on rat normal intestinal IEC-6 cells. After 72 h of incubation with the polyphenol extract IC_{50} values were 29 µg/mL and 44 µg/mL for Caco-2 and HT-29, respectively. Hence, for those cell lines IC_{50} was reached at higher concentrations of the extract than in the case of DU 145 and MDA-MB-231. On the other hand, for normal IEC-6 cells IC_{50} was not reached within the concentration range chosen by Gorlach et al. [15], as in the case of our studies with normal prostate PNT1A cells. According to a paper by Jaszewska et al., among three extracts from evening primrose seeds obtained using different solvents, the highest antiproliferative activity towards melanoma HTB-140 cells was observed for an aqueous-ethanolic extract (IC50 = 72.4 µg/mL) [20]. It should be emphasized that we extracted polyphenols from defatted seeds obtained from the same source as in the study by Jaszewska et al. [20], namely, a pharmaceutical company belonging to the Adamed Group (Agropharm S.A., Tuszyn, Poland). Furthermore, we used a 70% aqueous solution of ethanol as an extraction solvent. Therefore, one could conclude that the differences in the activities of evening primrose extracts reported in the above-mentioned papers and in the present work may result from the use of different cell lines, viability assays and extraction conditions (particularly the solvent used). As far as the stimulation of MDA-MB-231 cell viability is concerned, it was observed

only at the lowest EPE concentrations (7.25 μg/mL and 14.5 μg/mL) after 24 h and 48 h of incubation (Figure 2). The observed stimulation may result from the hormetic effect, as polyphenols can exhibit antioxidative activities at low concentrations and pro-oxidative activities at high concentrations [28].

As cell invasion plays an essential role in cancer metastasis, in this work we also investigated the effects of EPE on the invasion ability of the chosen cell lines. EPE suppressed the invasiveness of the nontumorigenic SV40-immortalized human prostatic epithelial cell line (PNT1A), which is characterized by low invasiveness, and of a highly invasive human tumorigenic breast cell line (MDA-MB-231). The suppressive effect of EPE on PNT1A and MDA-MB-231 invasiveness was statistically significant and dose-dependent (Figure 3A, 3B and 3C). On the other hand, EPE did not inhibit DU 145 invasiveness in a statistically significant manner within the chosen concentration range.

As discussed in the literature, one of the strategies for suppressing migration and invasion of cancer cells (including prostate and breast cancer cells) consists in inhibition of the activities of type IV collagenases (MMP-2 and MMP-9) [3,8,37]. Therefore, we assessed the influence of EPE on the activities of those enzymes by means of zymographic analysis. The activities of MMP-2 and MMP-9 secreted to culture medium by normal prostate PNT1A cells decreased dose-dependently after 48 h of incubation with EPE. At EPE concentration of 29 μg/mL MMP-2 activity decreased 4-fold while MMP-9 activity was reduced 2-fold compared to the control (Figure 4A). Importantly, EPE inhibited MMP-9 activity in both cancer cell lines after 48 h of incubation in a dose-dependent manner. At EPE concentration of 14.5 μg/mL the decrease in MMP-9 activity was over 2-fold whereas at the concentrations of 21.25 μg/mL and 29 μg/mL the reduction in the activity of this enzyme was over 5-fold (Figure 4B). In the case of breast cancer cells the inhibition of MMP-9 activity by EPE was definitely stronger than for the prostate cancer cell line.

The results presented above indicate a high antimetastatic potential of EPE, since type IV collagenases degrade type IV collagen, the major component of basement membranes [12,19]. It should be emphasized that basement membrane degradation is a crucial stage in migration and invasion of cancer cells and, consequently, in the formation of distant metastases [11,40]. Another team also reported inhibition of the activities of two enzymes, namely, aminopeptidase N (APN) and neutral endopeptidase (NEP), by a polyphenol extract from evening primrose defatted seeds [10]. According to Kiss et al. [10], APN was inhibited with IC_{50} of 2.8 μg/mL and 2.9 μg/mL for an aqueous extract and a 30% isopropanolic extract, respectively. Those results are in accordance with the results of our study, as APN plays an important role in tumor invasion, metastasis and angiogenesis [41]. On the basis of our results presented above, one could conclude that through the inhibition of MMP-9 activity in MDA-MB-231 cells and as a result of reduction of both MMP-2 and MMP-9 activities in PNT1A cells, EPE may suppress the invasiveness of those cell lines.

Conclusions

The polyphenol extract obtained from evening primrose defatted seeds (a waste product of the pharmaceutical and cosmetic industries) reduced the viability of prostate cancer cells (DU 145) and breast cancer cells (MDA-MB-231); in contrast, it did not cause a decrease in the viability of normal prostate cells (PNT1A). EPE also suppressed the invasiveness of two cell lines, PNT1A and MDA-MB-231 (highly invasive breast cancer cells), in a dose-dependent manner. Furthermore, EPE exhibited an antimigratory, anti-invasive and antimetastatic potential towards prostate and breast cancer cells by inhibiting the activities of both type IV collagenases (MMP-2 and MMP-9). Our results indicate that polyphenols from evening primrose (*Oenothera paradoxa*) seeds might be used in dietary supplements with cancer chemopreventive activity in the future.

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