

EFFECT OF THE SELECTED PHENOLIC AND FLAVONOID COMPOUNDS OF BLACK PEPPER AND CARAWAY SEEDS ON PROSTATE CELLS

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Abstract: In this study, the effect of selected phenolic and flavonoid compounds of black pepper and caraway seeds on prostate cells (PNT1A, 22RV1 and PC3) was observed. Synthetic standards of 3,4-dihydroxybenzaldehyde and naringenin chalcone, identified previously by HPLC-MS in black pepper seeds extracts, and neochlorogenic acid and apigenin, identified in caraway seeds extracts, were applied. For the evaluation of the potential inhibitory effect of selected compounds on PNT1A, 22RV1 and PC3 cells, the clonogenic assay and the microscopic observation of cells were done. The results of clonogenic assay showed that phenolic compounds had the strongest inhibitory effect on 22RV1 and PC3 cells, while the flavonoid compounds had the strongest inhibitory effect on PNT1A cells.

Key Words: spices, clonogenic assay, phenolic compounds, flavonoid compounds, prostate cell lines

INTRODUCTION

Prostate cancer is the second cause of cancer death for men (Atabi et al. 2017). A number of studies which show the anti-carcinogenic effects of piperine (Samykutty et al. 2013), curcumin (Nakamura et al. 2002, Chendil et al. 2004, Wei et al. 2012) or capsaicine (Surh and Kundu 2011) in prostate cancer cells have been conducted. In the context of previous research our experiment was focused on the studying of anti-carcinogenic ability of selected phenolic and flavonoid compounds identified by HPLC-MS analyses in methanolic extracts of black pepper seeds and caraway seeds. Previously published studies were focused on anti-cancerogenic effect of piperine, originated from black pepper seeds, on prostate cancer cells (Ouyang et al. 2013, Samykutty et al. 2013). Regarding the effect of caraway seeds phenolic extracts on prostate cells, no studies have been published yet. The selection of the most common phenolic and flavonoid compounds in caraway and black pepper seeds was based on our previous experiment (Lackova et al. 2016). This experiment showed that the most abundant phenolic and flavonoid compounds in black pepper are 3,4-dihydroxybenzaldehyde and naringenin chalcone, respectively. In caraway seeds, the most abundant phenolic compound was identified a neochlorogenic acid, whereas the most abundant flavonoid compound was an apigenin (Lackova et al. 2016). 3,4-dihydroxybenzaldehyde has anti-inflammatory and antioxidant effects, decreased proliferation effect on human cancer and induced apoptosis properties (Banerjee et al. 2016). Naringenin chalcone has inhibitory effects on some cancer cells (Zhang et al. 2016). Neochlorogenic acid has demonstrated the antioxidant and chemopreventive activity in some cancer cells (Banerjee et al. 2016). Apigenin inhibits tumor growth and angiogenesis induced by different cancer cells (He et al. 2012). Nevertheless, the effect of these four compounds has not been investigated in the prostate cells yet.

Based on our previous study (Lackova et al. 2016), we decided to determine the effect of selected phenolic and flavonoid compounds on the prostate carcinoma cell lines. In this experiment,

prostatic cells were exposed to selected phenolic and flavonoid compounds followed by clonogenic assay and cell observation performed under a microscope in time-dependent manner.

MATERIAL AND METHODS

Selected phenolic (3,4-dihydroxybenzaldehyde, neochlorogenic acid) and flavonoid (naringenin chalcone, apigenin) compounds were used. Apigenin and neochlorogenic standards were purchased from Extrasynthese (Genay, France). Naringenin chalcone standard was purchased from Phytolab (Vestenbergsgreuth, Germany). Three types of prostatic cells, PNT1A (immortalization of normal adult prostatic epithelial cells), 22RV1 (androgen dependent) and PC3 (androgen independent) cells were used. All cell lines used in this study were purchased from Health Protection Agency Culture Collection (Salisbury, UK). PNT1A, 22RV1 and PC3 cell lines were cultured in RPMI-1640 medium with 10% fetal bovine serum, supplemented with penicillin (100 U/ml) and streptomycin (0.1 mg/ml). Cells were then harvested, washed four times with PBS, pH 7.4, and counted using Countess IIFL Automated Cell Counter (Life Technologies, Carlsbad, CA). Other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA) in ACS purity.

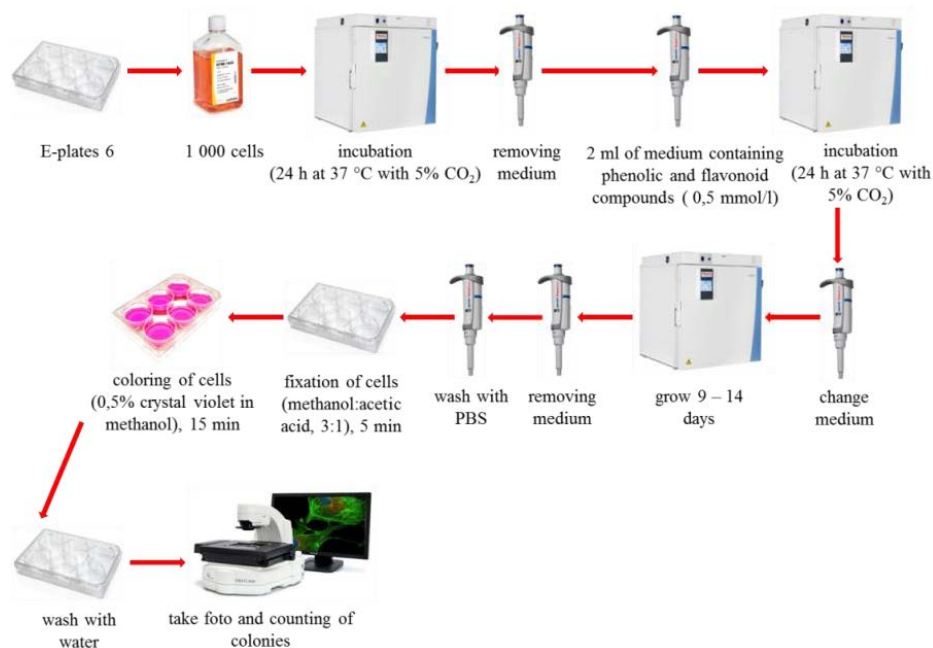
The preparation of samples containing phenolic and flavonoid compounds for observation of cells under a microscope (EVOS FL Auto Cell Imaging system, ThermoFisher Scientific, USA) was performed at 0, 1, 3, 6 and 12 hours of treatment (Figure 1). Images at a 400 μm magnification were obtained. Experiments were performed in duplicate.

Figure 1 Scheme of samples preparation for a microscopic observation



After observation of cells under a microscope, a clonogenic assay was performed (Figure 2). Cells were washed with Milli-Q water. Images were obtained with Canon EOS 650D (Canon, Óta, Japonsko). Experiments were performed in duplicate.

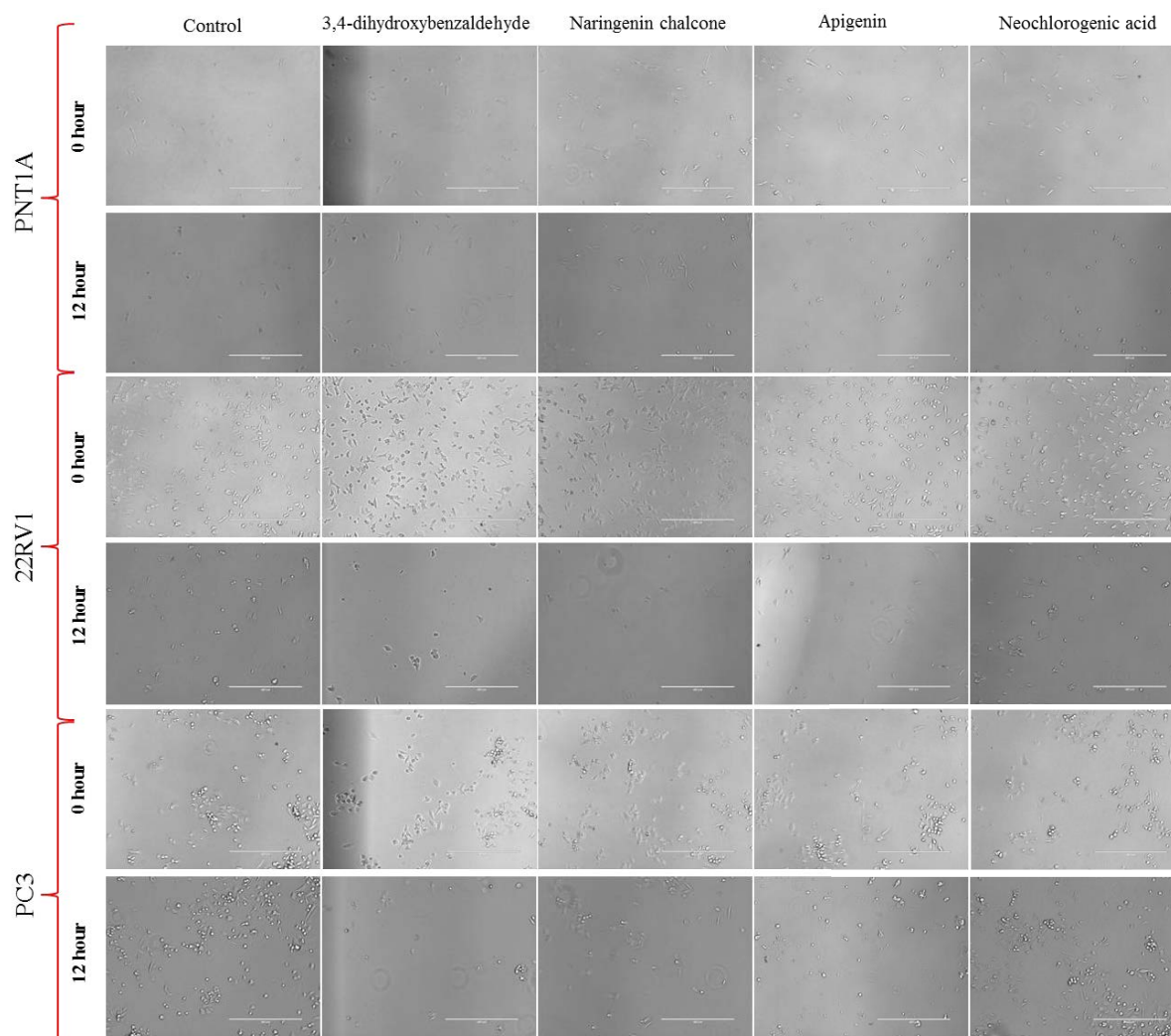
Figure 2 Scheme of samples preparation for a clonogenic assay



RESULTS AND DISCUSSION

The results of cells observation under a microscope (Figure 3) showed that PNT1A cells did not exhibit significant changes in growth over the 12-hour period compared to controls. PNT1A cells were growing very slowly in control samples. Nevertheless, different results were obtained for 22RV1 and PC3 cells when compared with PNT1A cells. The selected phenolic and flavonoid compounds have caused an inhibitory effect on 22RV1 and PC3 cells. In both cases, there was a gradual accumulation of cells and their inhibition compared to the control at time 0 hours.

Figure 3 Results of observation of cells under a microscope from 0 to 12 hours

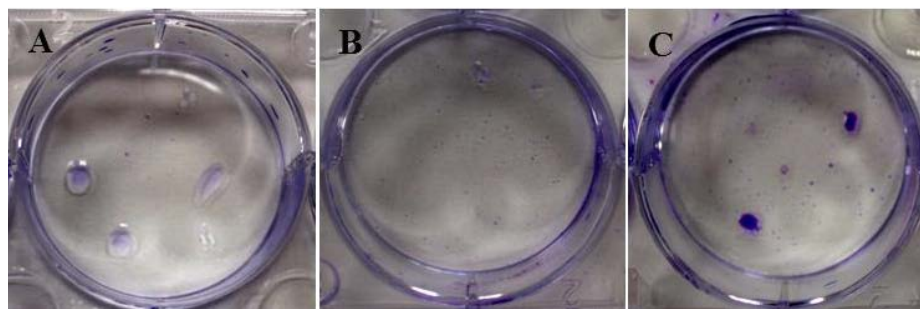


A clonogenic assay assesses the number of colonies growing after the treatment with the test compound, in our case with selected phenolic and flavonoid compounds. In all cell lines, the reduction of the number of colonies after the treatment with phenolic and flavonoid compounds was observed in comparison to control cell lines. The highest number of colonies, compared with control, was observed for PNT1A cells treated with naringenin chalcone. Conversely, the lowest number of colonies was observed when apigenin was applying, compared with control. For 22RV1 cells, the highest number of colonies was observed after the application of 3,4-dihydroxybenzaldehyde, and the lowest number of colonies was observed after the application of neochlorogenic acid, compared with control. For PC3 cells, the results were similar as in the case of 22RV1 cell lines. The highest number of colonies, compared with control for PC3 cells, was observed when naringenin chalcone was applied, while the lowest number of colonies was observed after the treatment with neochlorogenic acid.

Table 1 Results of clonogenic assay for PNT1A, 22RV1 and PC3 cells

Compounds	PNT1A cells		22RV1 cells		PC3 cells	
	Number of colonies*	%	Number of colonies*	%	Number of colonies*	%
Control	21.5	100	132.5	100	164.5	100
3,4-dihydroxybenzaldehyde	2.5	12	44.5	34	40.5	25
Naringenin chalcone	19.0	88	31.0	23	46.5	28
Apigenin	1.5	7	34.0	26	31.5	19
Neochlorogenic acid	3.0	14	2.5	2	24.5	15

Legend: * average of two measured values

Figure 4 Growing colonies in controls (A) for PNT1A cells, (B) for 22RV1 cells, (C) for PC3 cells

CONCLUSION

The aim of the experiment was to investigate inhibitory effect of selected phenolic and flavonoid compounds of black pepper and caraway seeds on three prostate cells (PNT1A, 22RV1 and PC3). From black pepper, 3,4-dihydroxybenzaldehyde and naringenin chalcone were used. From caraway seeds, neochlorogenic acid and apigenin were used. The results of observation of cells under a microscope showed that all four applied compounds had inhibitory effect on all cell lines used in the study. The results of clonogenic assay showed that the lowest number of colonies was observed for PNT1A treated with apigenin compared with control. For 22RV1 and PC3 cells, the lowest number of colonies compared with control was observed after the treatment with neochlorogenic acid.

This data serves as a pilot study for a larger experiment evaluating the effect of 3,4-dihydroxybenzaldehyde, neochlorogenic acid, naringenin chalcone and apigenin on prostate cell lines.

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