

# Tumour cell growth-inhibiting properties of water extract isolated from heated potato fibre (Potex)

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**Abstract:** Commercially available potato fibres are used as a food supplement which greatly improves the texture and stability of food. Food enriched with potato fibre is often cooked, baked and roasted. Such treatment creates new compounds with potential new biological activities. The aim of the study was an *in vitro* evaluation of the anticancer activity of water extract obtained from heated potato fibre (Potex). An antiproliferative effect of heated Potex extract is demonstrated in all tested tumour cell cultures, including rhabdomyosarcoma-medulloblastoma (TE671), glioma (C6), breast (T47D), colon (HT-29) and lung (A549) carcinoma. The study also showed that the tested extract produced remarkable morphological changes in tumour cells, decreased cancer cell motility, and induced apoptotic cell death. What is essential is that the applied concentrations of heated Potex extract did not influence the viability of normal human skin fibroblasts (HSF), rat oligodendrocytes (OLN-93) and mouse neurons. The potential role of heated potato fibre as a dietary chemopreventive is therefore suggested.

**Key words:** Potato fibre, Potex, anticancer activity, apoptosis, chemoprevention

## INTRODUCTION

Fibre has recently attracted attention as an important diet component responsible for health maintenance and disease prevention. It has been shown that dietary fibre elicits beneficial effects on human large bowel function, including increased faecal weight, shortened transit time, dilution of colonic contents, stimulation of colonic microbial growth, changes in nitrogen and bile acids metabolism, as well as absorption of organic and inorganic substances [1]. It also contributes to other processes, such as stabilising glucose and cholesterol levels, as well as lipid metabolism and antioxidant status improvement [2-4].

An increasing amount of data suggests that fibre deficiency is associated with increased risk of coronary heart disease [5], diabetes, colorectal adenoma and cancer [6-8]. Commercially available potato fibre has been found to be suitable for many foodstuff applications. As a stable raw material, potato fibres are used as dietary supplements and fat replacements for improving the texture and stability of food [9-12]. The potato fibre preparation Potex is a natural product consisting of dietary fibre (70%), starch, water (8.7%), proteins (5%), ash (4%) and fat (0.3%) (Promotion materials by Lyckeby Stärkelsen Food & Fibre AB, Kristianstad, Sweden, www.food.lyckeby.com). Food enriched by potato fibre is often cooked, baked or roasted, and through Maillard reaction during these processes several products are formed, among others, brown polymers known as melanoidins. Melanoidins derived from different sources, such as roasted coffee and different kinds of bread have been shown to

exhibit antiradical activity [13, 14], probiotic and antibacterial activity, promoting thereby the growth of *Bifidobacteria* strains and inhibiting *Helicobacter pylori* growth [15, 16].

At present, chemoprevention is defined not only as an application of natural or synthetic agents to suppress, arrest or reverse carcinogenesis in its early stages, but also to cure cancer [17, 18]. The term 'chemopreventive' has also been assigned to life style, including correct diet and nutritional habits [17]. Numerous food-derived chemopreventive agents are presently under intensive investigation, including *in vitro* systems, animal models and molecular analysis [19-24]. Dietary chemopreventives in active clinical trials include vitamins (vitamin D, folic acid), minerals (calcium, selenium), carotenoids (lycopene), flavonoids (genistein), and phenolic acids (resveratrol, curcumin, epigallocatechin-3-gallate) [17]. In this area, fibre is also considered as a potential dietary chemopreventive agent [25-27]. Unfortunately, there is a lack of information about the direct influence of native or processed fibre on cancer growth. Therefore, the objective of the present study was to examine the *in vitro* effects of water extract prepared from heated potato fibre (Potex). The influence on tumour cells proliferation, motility and death, as well as toxicity in normal cells was studied.

## MATERIALS AND METHODS

**Preparation of heated Potex.** Potato fibre (Potex) was obtained from Culinar, Sweden. 10 g of Potex was heated at 170°C for 2 hours, then suspended in 200 ml of distilled water. The mixture was incubated on a magnetic stirrer at 4°C for 24 hours. Next, the suspension was centrifuged (5,000 rpm/min.) for 30 min. and the supernatant collected (150 mL). After

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centrifugation, 50 ml of supernatant was vacuum evaporated, resulting in 0.7 g of a dark brown substance designated as PP170. Stock solution (10 mg/mL) was prepared in culture medium (1:1 mixture of DMEM and Nutrient mixture F-12 Ham (Sigma Chemicals, St. Louis, MO, USA) + 10% Fetal Bovine Serum – FBS, (Sigma). The following working solutions were applied: 100, 250, 500 and 1,000 µg/mL.

**Cell culture.** Human rhabdomyosarcoma/medulloblastoma (TE671) was obtained from the European Collection of Cell Cultures, Centre for Applied Microbiology and Research, Salisbury, UK. Human breast carcinoma (T47D) was obtained from the Department of Human Genetics, Medical University, Lublin, Poland. Human colon adenocarcinoma (HT-29) and human lung carcinoma (A549) were obtained from the Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wrocław, Poland. Rat glioma (C6), rat oligodendrocytes (OLN-93) and mouse embryonal carcinoma (P19) were obtained from the Department of Neonatology, Charité-Virchow Clinics, Humboldt University, Berlin, Germany. Human skin fibroblasts (HSF) were a laboratory strain obtained by the outgrowth technique from skin explants of young persons. The following culture media purchased from Sigma were applied: DMEM (C6, HSF), 1:1 mixture of DMEM and Nutrient mixture F-12 Ham (TE671, T47D, HT-29, OLN-93). All media were supplemented with 10% FBS (Sigma), penicillin (100 U/mL) (Sigma) and streptomycin (100 µg/mL) (Sigma). The cultures were kept at 37°C in a humidified atmosphere of 95 % air and 5 % CO<sub>2</sub>.

Neuronal cell cultures were prepared from retinoic acid-induced neural differentiation of P19 mouse embryonal carcinoma cells. P19 cells were maintained in MEM Alpha Medium (Gibco, Paisley, UK) supplemented with 10 % FBS, 100 U/mL penicillin and 100 µg/mL streptomycin. Neuronal differentiation was induced in  $\alpha$ -MEM culture medium supplemented with 5 % FBS and 0.5 µM of retinoic acid (Sigma). The resulting cell aggregates were dispersed during incubation with 0.25 % Trypsin-EDTA solution supplemented with 50 µg/mL of DNase I. Neurons were plated on poly-L-lysine coated 96 multiwell plates (Nunc, Roskilde, Denmark). The following culture medium was applied: Neurobasal Medium (Gibco) with 2 % of B-27 supplement (Gibco), 100 U/mL penicillin and 100 µg/mL streptomycin. Neuronal cultures were incubated at 37°C in humidified 95 % air and 5 % CO<sub>2</sub> atmosphere. Culture medium was changed every 3 days until day 10, at which point the neurons were subjected to experiments.

**Proliferation assay.** Tumour cell proliferation was assessed by 2 methods: MTT and BrdU incorporation assay. In the MTT assay, tumour cells were plated on 96-well microplates (Nunc) at a density of  $0.5 \times 10^4$  (C6),  $1 \times 10^4$  (TE671, T47D, A549) and  $3 \times 10^4$  (HT-29). Next day, the culture medium was removed and the cells exposed to serial dilutions of tested extract in a fresh medium. Cell proliferation was assessed after 96 hours by means of the MTT method in which the yellow tetrazolium salt (MTT) is metabolized by viable cells to purple formazan crystals. Tumour cells were incubated for 3 hours with MTT solution (5 mg/mL). Formazan crystals were solubilized overnight in SDS buffer (10 % SDS in 0.01 N HCl) and the product quantified spectrophotometrically by measuring absorbance at 570 nm wavelength using an E-max Microplate Reader (Molecular Devices Corporation, Menlo Park, CA, USA). In BrdU assay, tumour cells (TE671 and T47D) were

plated on 96-well microplates at a density of  $2 \times 10^4$ . Next day, the culture medium was removed and the cells were exposed to serial dilutions of Potex extract in fresh medium. Cell proliferation was quantified after 48 hours by measurement of BrdU incorporation during DNA synthesis (Cell Proliferation ELISA BrdU, Roche Diagnostics GmbH, Penzberg, Germany). Tumour cells were incubated with 10 µM BrdU for 2 hours. Cells were subsequently incubated with FixDenat solution for 30 min. and then exposed to monoclonal anti-BrdU antibodies conjugated to peroxidase. Colour reaction was developed by adding TMB substrate solution, and terminated by the addition of 1 M H<sub>2</sub>SO<sub>4</sub>. The absorbance was measured at 450 nm wavelength using an E-max Microplate Reader.

**Assessment of cell death.** Measurement of cell death was performed by using a Cell Death Detection ELISA<sup>PLUS</sup> kit (Roche Diagnostics). The assay is based on a quantitative sandwich-enzyme-immunoassay-principle using mouse monoclonal antibodies directed against DNA and histones, respectively. This allows the specific determination of mono- and oligonucleosomes in the cytoplasmatic fraction of cell lysates. Tumour cell cultures (TE671, T47D) growing on 96-well microplates were subjected to tested extract (100, 250, 500 and 1,000 µg/mL) for 24 hours, after which the supernatants were removed and cells lysed with 200 µL of lysis buffer for 30 min. Subsequently, cell lysates were centrifuged at  $200 \times g$  for 10 min. and 20 µL of the samples were carefully transferred into the streptavidin-coated 96-well microplate. The immunoreagent (80 µL) containing anti-histone-biotin and anti-DNA-POD mouse monoclonal antibody was added and incubated under gentle shaking (300 rpm) for 2 hours at 20°C. The solution was removed by tapping, each plate well rinsed 3 times with 250 µL of incubation buffer, and finally, 100 µL per well of substrate solution (ATBS) was applied and incubated at room temperature for 15 min. on a plate shaker (250 rpm) until sufficient colour developed. Absorbance was measured at 405 nm wavelength using an E-max Microplate Reader.

**Cell migration assessment.** Tumour cell migration was assessed by means of the wound assay model [28]. Tumour cells (C6, TE671) were plated at  $1 \times 10^6$  cells on 4 cm diameter culture dishes (Nunc). Next day, the cell monolayer was scratched by pipet tip (P300), the medium and dislodged cells aspirated, and the plates rinsed twice with PBS. Next, fresh culture medium was applied and the number of cells migrated into the wound area after 24 hours was estimated in control, and cultures treated with Potex extract (100 and 250 µg/mL). Plates were stained by the May-Grünwald-Giemsa method. Observation was performed by Olympus BX51 System Microscope (Olympus Optical CO., LTD, Tokyo, Japan) and micrographs prepared by analySIS<sup>®</sup> software (Soft Imaging System GmbH, Münster, Germany). Cells migrated to the wound area were counted on micrographs, and the results expressed as a mean cell number migrated to the selected 50 wound areas taken from 4 micrographs.

**Light microscopy.** Tumour cells were plated on Lab-Tek Chamber Slide (Nunc) at a density  $2 \times 10^4$  cells/mL (C6, TE671, T47D) and  $4 \times 10^4$  cells/mL (HT-29). Next day, the culture medium was replaced with fresh medium containing the vehicle and heated Potex extract (500 and 1,000 µg/mL). Cultures were allowed to grow for 48 hours, and subsequently stained by the May-Grünwald-Giemsa method. Observation

was performed by Olympus BX51 System Microscope, and micrographs prepared by means of analySIS® software.

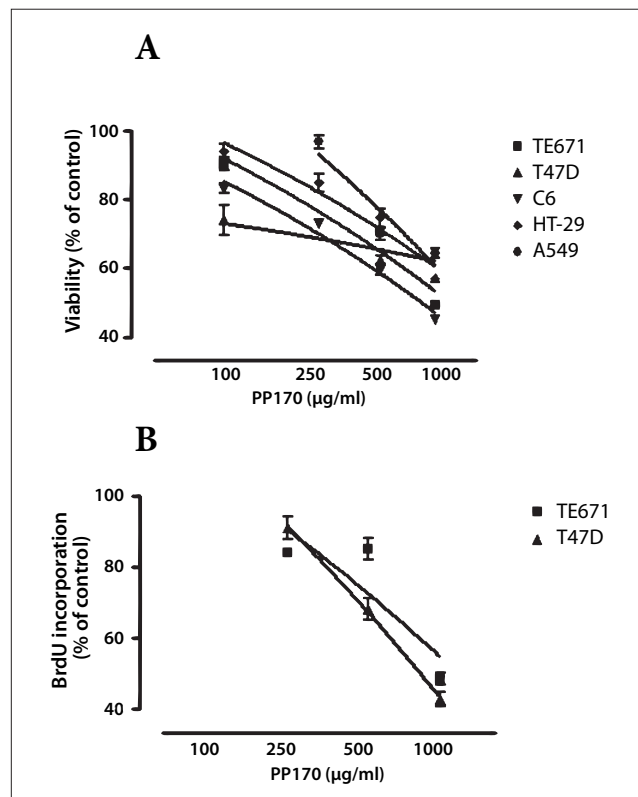
**Cytotoxicity assay.** A cytotoxicity detection kit based on measurement of lactate dehydrogenase (LDH) activity was applied (Tox-7, Sigma). This assay is based on the reduction of NAD by the action of LDH released from damaged cells. The resulting NADH is utilized in stoichiometric conversion of a tetrazolium dye. The resulting coloured compound is measured spectrophotometrically. Human skin fibroblasts (HSF) and rat oligodendrocytes (OLN-93) were plated on 96-well microplates at a density  $1 \times 10^5$ . Next day, the culture medium was removed and the cells subjected to tested Potex extract diluted in a fresh culture medium with a reduced amount of FBS (2%). The same treatment was applied in the case of neuronal cell cultures. Culture supernatants were collected after 24 hours and incubated with substrate mixture for 30 min. at room temperature, in the dark. Finally, the reaction was terminated by the addition of 1N HCl and the colour product quantified spectrophotometrically at 450 nm wavelength using an E-max Microplate Reader.

## RESULTS

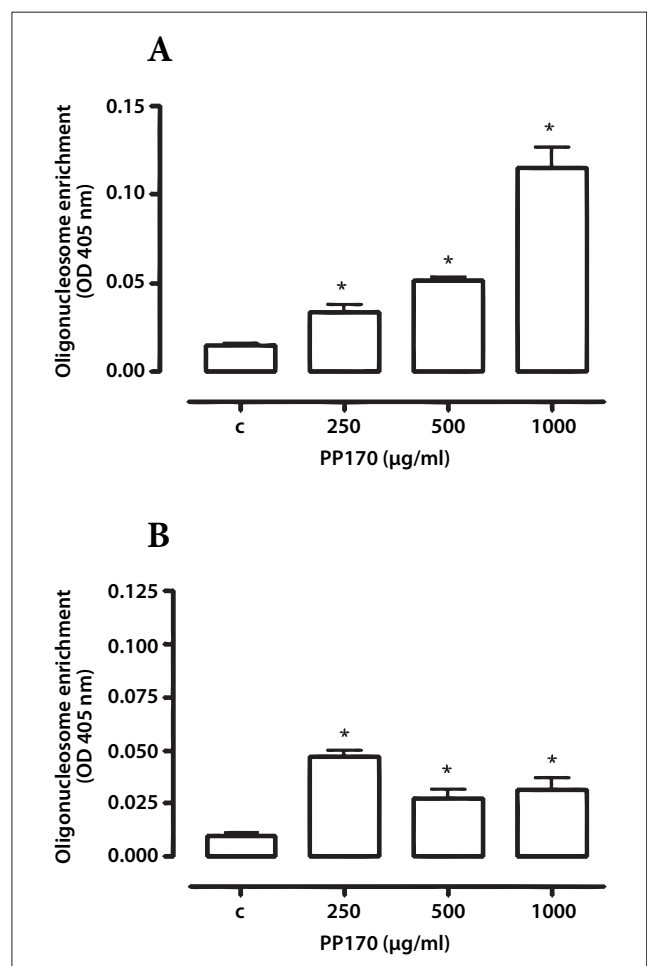
In the preliminary experiments, a time course study was performed which revealed that Potex extract obtained after

2 hours heating possessed the highest antiproliferative activity (data not shown). Therefore, this extract was tested in a further series of experiments. The antiproliferative activity of heated Potex was assessed in 5 cancer cell lines: human rhabdomyosarcoma/medulloblastoma (TE671), human breast carcinoma (T47D), human colon adenocarcinoma (HT-29), human lung carcinoma (A549), and rat glioma (C6). Cells were exposed either to the culture medium (control) or the tested extract (100, 250, 500, 1,000  $\mu\text{g}/\text{mL}$ ) for 96 hours. Proliferation of tumour cells decreased in a concentration-dependent fashion, as measured by means of the MTT assay (Fig. 1A). Threshold concentrations of tested extract required to elicit a significant effect were as low as 100  $\mu\text{g}/\text{mL}$  (T47D, C6) and 250  $\mu\text{g}/\text{mL}$  (TE671, HT-29) and 500  $\mu\text{g}/\text{mL}$  (A549). The antiproliferative effect of heated Potex was attributed to decreased cell division, as determined by measurements of incorporation of BrdU during DNA synthesis (Fig. 1B).

Cell Death ELISA was applied to evaluate whether the tested extract induces apoptotic cell death. In this assay, the increase of immunoreactive cytosolic oligonucleosomal fragments as a characteristic marker of DNA degradation was measured. Exposure to heated Potex extract triggered significant apoptotic cell death in TE671 (Fig. 2A) and T47D (Fig. 2B) cells, as indicated by oligonucleosome enrichment.

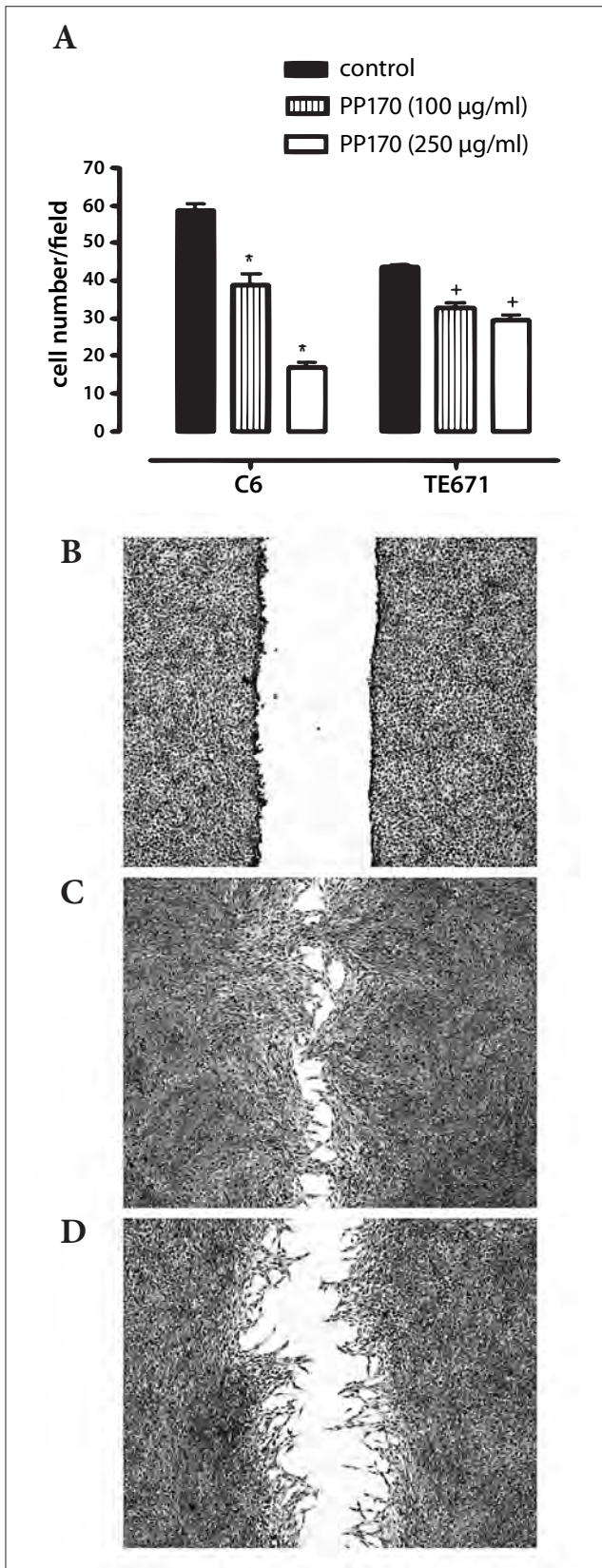


**Figure 1** (A) Antiproliferative activity of heated Potex (MTT assay). TE671, T47D, C6, HT-29 and A549 cells were grown in culture medium only (control) and in the presence of PP170 (100-1000  $\mu\text{g}/\text{mL}$ ) for 96 hours. The data represent mean normalized % of cell viability  $\pm$  SEM of 5-6 trials and were analyzed by means of linear regression. (B) The anti-proliferative effect of heated Potex was attributed to decreased cell division. TE671 and T47D cells were grown in culture medium only (control) and in the presence of PP170 (100-1000  $\mu\text{g}/\text{mL}$ ) for 48 hours. BrdU incorporation was used as a marker of cell division. The data represent the mean normalized % of BrdU incorporation  $\pm$  SEM of 4-5 trials and were analyzed by means of linear regression.

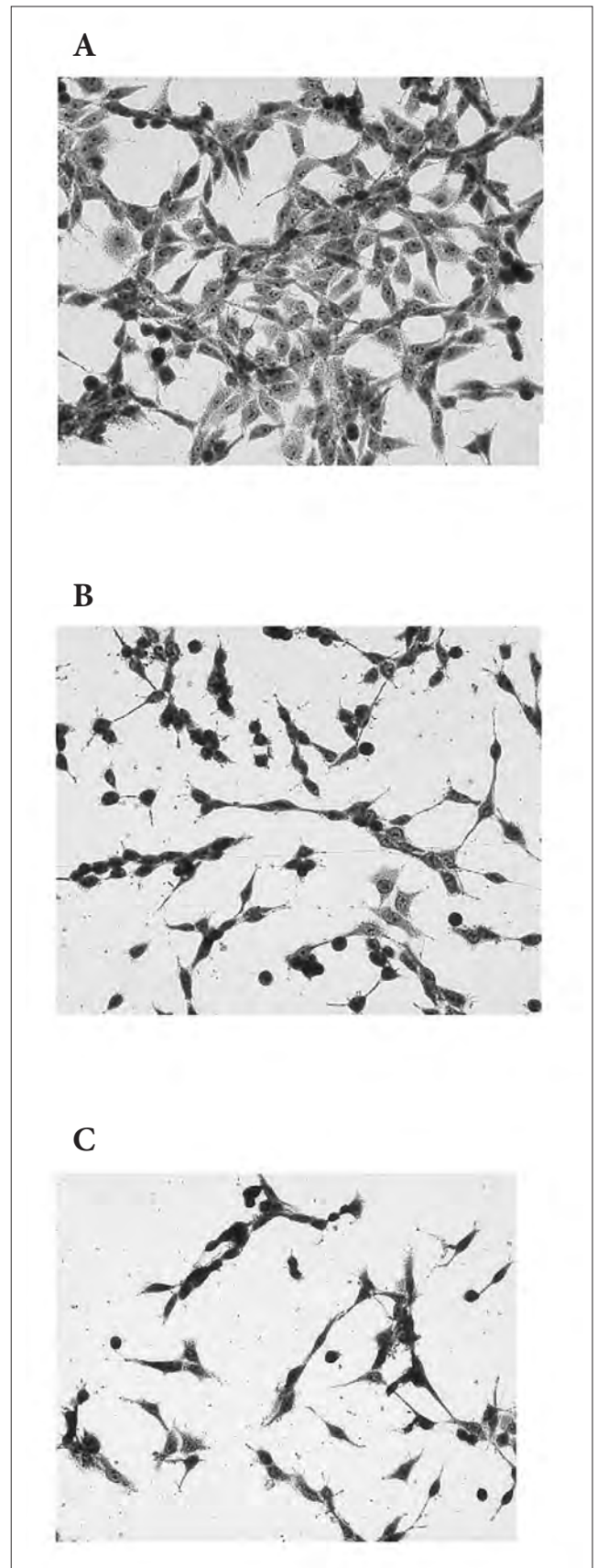


**Figure 2** Heated Potex induces apoptotic cell death in TE671 (A) and T47D (B) cells. Enrichment of oligonucleosome fragments in the absence (c) and following 24 hours of PP170 exposure (250-1000  $\mu\text{g}/\text{mL}$ ). The data represent mean normalized optical densities  $\pm$  SEM of 2-4 trials, \*at least  $p < 0.05$  vs. control, one-way ANOVA, post-test: Tukey.

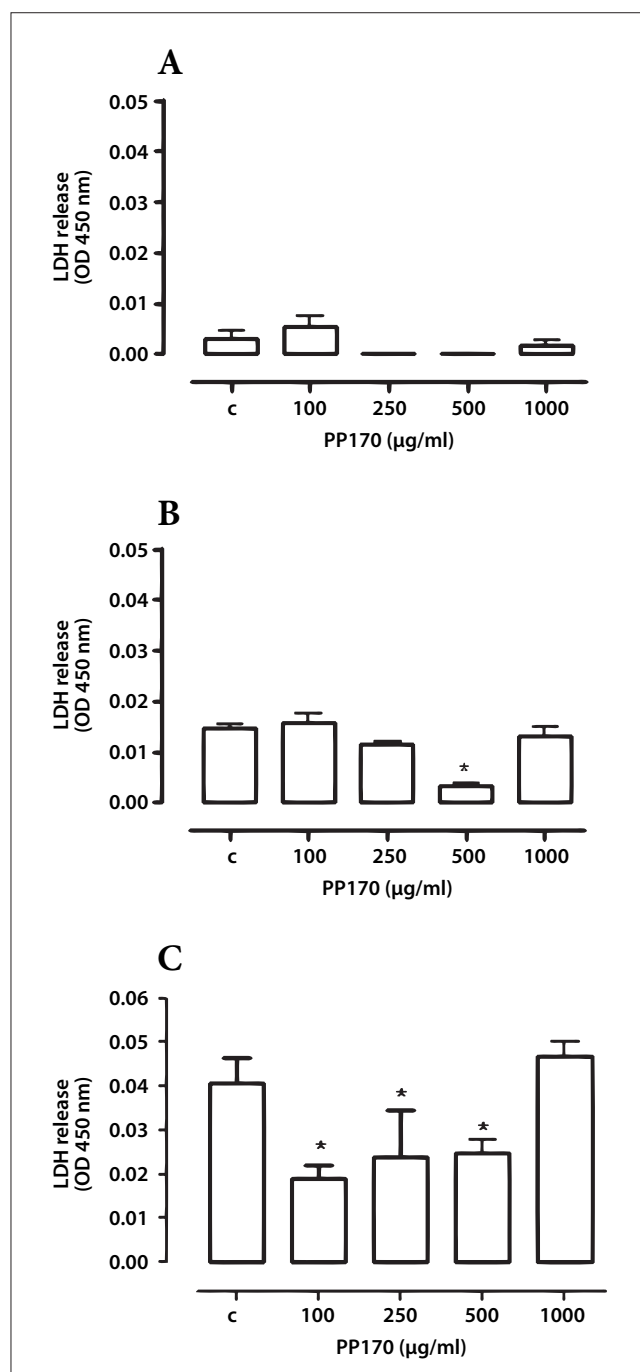




**Figure 3** Effect of Potex extract on tumour cell motility. Scratched monolayers of tumour cells (C6, TE671) were incubated for 24 hours alone or in the presence of PP170 (100 and 250 µg/mL). Tumour cells which migrated to the wound area were counted (A). Micrographs show wound assay of glioma C6 cells; wound (B), cell migration after 24 hours in control culture (C) and following exposure to 250 µg/mL of PP170 (D). Magnification 40x. The results are expressed as the mean number of cells migrated per field of the wound area ± SEM of 50 measurements (\*).  
+ At least  $p < 0.05$  vs. control, one-way ANOVA, post-test: Tukey.



**Figure 4** Effect of heated Potex extract on tumour cell morphology. Light micrographs of glioma C6 cells under control conditions (A) and following exposure to PP170 500 µg/mL (B) and 1000 µg/mL (C). Tumour cells exposed to Potex extract display dose-dependent shrinkage and an elongated appearance. Magnification 100x.



**Figure 5** Cytotoxicity of Potex extract in normal cells. Cell death was quantified after 24 hours in HSF (A), OLN-93 cells (B) and neurons (C) by measuring LDH release in the absence (c) or in the presence of PP170 (100-1000 µg/mL). Data represent mean optical densities  $\pm$  SEM of 4-6 trials.

\*At least  $p < 0.05$  vs. control, one-way ANOVA, post test: Tukey.

To test the effect of heated Potex on tumour cell motility, glioma (C6) and rhabdomyosarcoma/ medulloblastoma (TE671) cells were exposed to either culture medium or PP170 (100 and 250 µg/mL) for 24 hours. Wound assay revealed that in cultures exposed to the tested extract, significantly fewer cells migrated to the wound area (Fig. 3A). This effect was dose dependent and much more pronounced in glioma C6 cell culture. Micrographs showing inhibited glioma C6 cells migration following heated Potex exposure are presented in Figure 3B, C and D.

In order to show the influence on tumour cell morphology, cells were exposed to the tested extract at concentrations of 500 and 1,000 µg/mL. Light microscopy revealed that heated Potex induced pronounced changes in tumour cell morphology. In C6 (Fig. 4) and TE671 cells (not shown) it produced dose dependent shrinkage and induced elongated cell appearance. In HT-29 cells the anticancer effect was expressed as a massive colony shrinkage and cell degeneration. A less pronounced effect was observed in the case of T47D cells (data not shown).

To evaluate the effect on normal cell viability, human skin fibroblasts (HSF), rat oligodendrocytes (OLN-93) and mouse neurons were subjected to increasing doses of PP170. LDH assay revealed that the tested extract was not toxic to HSF (Fig. 5A), OLN-93 (Fig. 5B) and neurons (Fig. 5C). Moreover, a significant decrease of LDH release at concentrations of 100-500 µg/mL was observed in neuronal cell culture (Fig 5C), and at 500 µg/mL in OLN-93 cell culture (Fig. 5B).

## DISCUSSION

To date, anti-cancer activity of heat processed fibre has not been researched. Our study reveals for the first time that water extract obtained from heated potato fibre exerts an antiproliferative and pro-apoptotic activity against cancer cell lines of different origin. Apoptosis is one of the most potent mechanisms of anti-cancer defence. Many chemopreventive agents, including those of dietary origin, have been found to induce apoptotic pathways in cancer cells in both *in vitro* and *in vivo* models [29, 30]. Even more interesting than the growth inhibition induced by heated Potex was the impairment of cancer cell motility. The ability of tumour cells to migrate is one of the hallmarks of tumour metastatic potential. Targeting cancer metastasis has the highest priority in cancer therapy, since metastatic disease and not local tumour growth determines the mortality of most patients. It was detected that the tested extract at low concentrations significantly reduced motility of glioma (C6) and medulloblastoma (TE671) cells *in vitro*. On the other hand, it is of the highest significance that the potential dietary chemopreventive agent taken regularly for a long period of time does not affect normal tissues. In the presented experiments, heated Potex extract exhibited low cytotoxicity against normal skin fibroblasts and rat oligodendrocytes, when measured by sensitive, LDH release test. Moreover, the observed neurotrophic effect in neuronal cultures suggests a beneficial activity and opens a new area for future investigations. The nervous system is especially vulnerable during anti-cancer therapy, and many side effects have been described in this respect [31, 32]. Further studies are needed to verify the hypothesis concerning the potential role of heated potato fibre in neuroprotection and/or neurodegenerative disease prevention.

The active components of heated Potex extract remain to be elucidated. Heat treatment of food produces the formation of the flavour compounds and brown pigment. The brown and high molecular weight products of the Maillard reaction are defined as melanoidins [33]. Melanoidins are formed by interaction between reducing sugars and compounds possessing free amino groups, such as free amino acids and the free amino groups of peptides [34]. The anti-cancer activity of some melanoidins have already been described. Melanoidins extracted from polysaccharide immunomodulator – PSK,



had a direct growth inhibitory effect on HCT-15 and AGS cells derived from human colon and stomach cancer [35]. Melanoidins extracted from soy sauce were shown to block cell cycle progression in S and G2/M phase in stomach and colon cancer cells [36]. It has already been detected that coffee melanoidins may complex other substances such as polyphenols [37]. Chlorogenic acid, a polyphenol present in potatoes, revealed chemopreventive activity in glioma cells by inhibition of microsomal glucose-6-phosphate translocase, an enzyme responsible for glioma invasiveness [38]. The analysis of heated Potex extract towards melanoidin and polyphenol presence will therefore be the subject of further studies. Although, as mentioned above, much remains to be researched, it may be supposed that our findings pose some new challenges for researchers and the food industry. It is believed that after successful complementary studies, heated potato fibre (Potex) may become a new candidate as a dietary chemopreventive agent.

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