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Red cabbage anthocyanin extract alleviates copper-induced cytological disturbances in plant meristematic tissue and human lymphocytes

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Abstract Red cabbage is a source of health beneficial substances with antioxidant and antigenotoxic properties. HPLC analysis specifying the content of the investigated extract indicated that mainly anthocyanins (ATH) were responsible for its abilities. Cytological research was conducted with two experimental models: plant tissues—meristematic cells of *Vicia faba*, and animal tissue elements—human lymphocytes. Positive influence of ATH extract on mitotic activity of *Vicia* cells exposed to Cu^{2+} stress, and inhibitory effect of ATH on cytotoxic actions of Cu^{2+} on lymphocytes were demonstrated. In all experimental series with ATH application in combinations with Cu^{2+}, mitotic index (MI) were higher than those obtained for only Cu^{2+} stressed tissues. Preincubation in ATH before Cu^{2+} stress had the best effect. Similarly, after ATH applications in all tested series decrease in frequency of micronuclei (MN) appearance was noticed in comparison with only Cu^{2+} stressed material. In the case of *Vicia* cells ATH acted effectively even applied after Cu^{2+} stress. It suggests that this ATH mixture not only prevents and limits but also heals the cytological injury caused by Cu^{2+} stress.

Keywords Anthocyanins · *Brassica oleracea rubrum* · Copper ions · Cytogenetic tests

Abbreviations

ATH	Anthocyanin
MI	Mitotic index
MN	Micronuclei
ROS	Reactive oxygen species

Introduction

Copper is a transition metal able to cycle between two red–ox states. Virtually all organisms require copper as a catalytic cofactor for biological processes such as respiration, oxidative stress protection, pigmentation. However, this metal also participates in redox reactions that generate toxic species such as hydroxyl radical, which damage the most important structures in cells. (Fernandes and Henriques 1991).

Various plant species react differently to Cu^{2+} excess but differences in responses seem to result not only from its concentration but also from plants ability to the antioxidative protection against negative consequences of heavy metal stress. The metal toxicity resulting from the oxidative state may be allayed by enzymatic and non-enzymatic antioxidative systems

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including phenolic compounds (PhC), proline, tocopherols and polyamines (Mittler 2002; Grace 2005; Górecka et al. 2007).

Stress-induced changes of phenylpropanoid metabolism are relatively well characterised, but our knowledge of heavy metal-dependent modifications in phenylpropanoid metabolism, in their composition and accumulation is much less documented. It has been observed that these compounds are constitutively expressed in higher plants and can effectively prevent oxidative stress caused by unfavourable environmental factors e.g. low temperature (Janas et al. 2002), pathogen infections (Treutter 2006), UV radiation (Bieza and Lois 2001). However, their metabolism may often be induced when plants are exposed to as well as heavy metals such as Cu^{2+} (Caldwell 2002; Sgherri et al. 2003; Jung et al. 2003; Skórzyńska-Polit et al. 2004; Ali et al. 2006; Górecka et al. 2007).

Many polyphenols have ideal structure and redox properties for free radical scavenging and can chelate transition metal ions thus preventing the formation of reactive oxygen species (ROS) (Montoro et al. 2004). Anthocyanins (ATH), plant polyphenols, a subclass of flavonoids, are water-soluble pigments responsible for the colours of flowers, fruit and other vegetable organs. It has been proposed that these compounds play an important role in photoprotection and photoinhibition in plants (Steyn et al. 2002). They are widespread in nature, innocuous and beneficial to human health (De-Xing 2003). ATH posses pharmacological properties protecting against cardiovascular diseases and are postulated as anti-mutagenic and anti-tumour factors (Gąsiorowski et al. 1997; Wang et al. 2000; Kong et al. 2003). The above effects have been related mainly to the antioxidant properties of ATH, as it was demonstrated with experiments both in vitro and in vivo (Boveris et al. 2001; Kong et al. 2003; Lazzé et al. 2003). They have been shown to be highly effective scavengers of most types of oxidising molecules such as various free radicals (Wang et al. 1997).

Cytogenetic part of presented experiments included research on exogenous ATH role in maintaining genetic stability in tissues exposed to high copper concentration. General roles of plant polyphenols in ensuring a stable genome were described by Ferguson (2001). We would like to verified the hypothesis about a positive antigenotoxic effect of a precisely defined group of phenolic compounds—anthocyanins extracted from red cabbage.

Research were carried out on two experimental models which allow for a wide estimation of the tested substances at cytological, cytogenetical and molecular levels both, in a plant tissue (*Vicia faba* test according to *International Programme of Chemical Safety of World Health Organisation* protocol (Grant 1994; Kanaya et al. 1994) and human tissue (in vitro culture of human peripheral blood lymphocytes recommended by *The Expert Panel of The International Workshop on Genotoxicity Test Procedures* (Albertni et al. 2000).

Inhibition of cell mitotic activity is connected with disturbances of basic processes in all cell cycle phases: G_1, S, G_2 and M. Mitotic index (MI) is a characteristic and stable value for particular tissues and organs. Changes in this value suggest influence of exogenous factors and let us preliminarily conclude about the type of action of these factors (Ma et al. 2005; Silva-Pereira et al. 2005; Sang et al. 2006).

The micronuclei (MN) tests in the root tip of *Vicia faba* and in vitro cultured lymphocytes have been widely used in biomonitoring genotoxicity both in water and soils (Ma et al. 1995; Minissi and Lombi 1997; Stopper and Müller 1997; Duan et al. 2000;) These tests were recommended by Micronuclei Assay Expert Panel at the Second International Workshop on Genotoxicity Test Procedures as a standard test in genotoxicity research (Humpage et al. 2000). Micronuclei are little structures containing chromatin but not connected with a cell nucleus. Mechanisms of micronuclei formation are complicated but their common feature is that they are visible only after cell division, even if a factor/mutagen acted during interphase or in early mitosis (Stopper and Müller 1997). Most frequently (in 60% of cases) micronuclei appear because of chromosome or chromatide breakage. Acentric fragments are not included into the new nucleus and stay in cytoplasm.

The aim of this study was to assess the ability of the ATH-rich extract from red cabbage leaves to modify the oxidative and cytotoxic effects of Cu^{2+} on plant and animal tissues. Two standard cytological tests: mitotic index (MI) determination and micronucleus test (MN) were done.

Materials and methods

Chemicals

To examine the effect of heavy metal stress on the tested models: *Vicia faba* seedlings or in vitro cultured human lymphocytes, copper was applied as $CuSO_4$ water solution added to the medium in the calculated amount to obtain the final concentrations indicated below for each model system. The stock solutions of the metal salts were prepared in distilled and then double-deionized water. For the control experiment Cu^{2+} was omitted.

All chemicals used in presented experiments were of analytical grade. All of them excepting Histofluid (Medlab, Poland) were purchased from Sigma Co. (Schnelldorf, Germany).

ATH extraction and purification

Fresh leaves of red cabbage (*Brassica oleracea* var. *capitata rubra*) were homogenised and extracted with the mixture: methanol/distilled water/HCl 1% (EMix: MeOH/H_2O/HCl, 50/50/1, v/v/w) and centrifuged (Gitz et al. 1998). Supernatant was collected as a crude red cabbage extract. To obtain dry matter, the extract was dried in a vacuum rotary evaporator in a water bath at 40°C. It condensed (30 mg ml^{-1}) solution with acidified 10% MeOH was prepared and than purified on high load C18 SPE mini-column (Alltech Assoc.) previously activated with MeOH. Using this method the samples (1.5 ml) were desalted with 0.01% aqueous HCl before removal of small phenolics by rinsing with ethyl acetate. Then the column was eluted with acidified MeOH gradients in the following order: 10, 20, 50 and 80%. Volume of the used solvents was always 5 ml per column ($\sim 3 \times$ sample volume).

Colourful fractions in 50% MeOH were collected HPLC analysed and used in the cytogenetic experiments as ATH source.

Screening method for ATH assay

Anthocyanin concentration (μM) in the extracts was determined spectophotometrically following cyanidin 3-glucoside as a standard and calculated using molar absorption coefficient $\varepsilon = 30$ mM^{-1} cm^{-1} at $\lambda = 525$ nm (Hodges and Nozzoillo 1996; Gitz et al. 1998).

HPLC analysis of the extract

Phenolic profiles in purified red cabbage extracts were determined using HPLC Knauer system equipped with UV–Vis detector and a Eurospher-100 C-18 column (25 cm × 4.6 mm; 5 μm). The binary mobile phase according to Dyrby et al. (2001) consisted of water/formic acid (90:10, v/v) (solvent A) and water/acetonitrile/formic acid (40:50:10, v/v/v) (solvent B). The flow rate was 1 ml/min and a total run time was 50 min. The system was run with a gradient programme: 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. Phenolics were divided into four subclasses and quantified on the basis of the maximum UV–Vis absorption of each group. The hydroxybenzoic acid derivatives were quantified at 280 nm and expressed as gallic acid equivalents, hydroxycinnamic acid derivatives at 320 nm as chlorogenic acid equivalents, flavonols at 360 nm as rutin equivalents, and anthocyanins at 520 nm as cyanidin 3-glucoside equivalents (Podsędek et al. 2006).

Plant growth conditions

Seeds of faba bean (*Vicia faba* L. ssp. *minor*) obtained from the Institute of Plant Cultivation and Acclimation (Sobiejuchy/Poland) were germinated according to Osiecka and Janas (1998). The seeds were placed in boxes with two layers of Wht 2 filter paper wetted with distilled water and germinated for 3 days at 25°C in darkness. Seedlings with 1.5–3 cm long roots were chosen for the tests.

Human lymphocytes in vitro culture

Lymphocytes were isolated from peripheral blood of healthy, non-smoking donors (the Blood Bank in Lodz, Poland) by density-gradient centrifugation (15 min, 300 g) with Histopaque-1077 (Sigma). Cultures were set up in 80% RPMI 1640 medium containing 15% inactivated foetal bovine serum, 1% penicillin/streptomycin solution, 1% phytohemagglutinin (added 24 h before application of the tested compounds) and placed at 37°C in a humid chamber

for 72 h (Błaszczyk 2006). The viability of the isolated lymphocytes was about 90–95%.

Cu^{2+} cytotoxicity test for Vicia

Mitotic Index (MI) wase used as a marker of Cu^{2+} cytotoxicity for *Vicia* meristematic cells. The MI for each variant was determined as the percentage of mitotic figures in 1,000 cells from the same root tip in at least four independent experiments (Glinska et al. 2007).

Changes in MI suggest influence of exogenous factors and let us preliminarily conclude about their type of action. Concentration of such a factor should generate stress but cannot be strongly cytotoxic or lethal in order to make continuation of experiments and investigations possible. Usually it is the concentration causing 50–60% inhibition of MI.

3-day old *Vicia faba* seedlings incubation was carried out with $CuSO_4$ water solutions at final concentrations: 0 (control), 0.5; 1.5; 2.5; 5; 10 mM, for 2 h at 25°C in darkness.

Cu^{2+} cytotoxicity test for lymphocytes

In vitro estimation of Cu^{2+} cytotoxicity for human lymphocytes was carried out using MTT [3(4, 5-dimethylthiazol-2-yl)2,5-diphenyltetrazolium] test (Kośmider et al. 2004). MTT test is based on the cleavage of the yellow dye MTT to purple formazan crystals by mitochondrial succinic dehydrogenase; this conversion only occurs in viable cells. Lymphocytes were seeded on a 96-weel microplate 24 h before Cu^{2+} treatment. The cells were incubated with different final concentrations of $CuSO_4$ solutions: 0 (control), 0.01; 0.02; 0.05; 0.07; 0.08; 0.1; 0.2; 0.5 mM, for 1 h. Than 15 μM of MTT was added to each well, after 2 h formazan crystals were dissolved in 20% SDS (sodium dodecyl sulphate)/50% DMF (*N*,*N*-dimethyl-formamide). After further 24 h, the absorbance at 580 nm was measured. Three independent experiments were performed for each Cu^{2+} concentration variant. The IC_{50} values (Inhibitory Concentration 50%) were estimated on the basis of survival curves.

Cytogenetic assay protocol for Vicia faba meristematic cells

The seedlings were incubated with $CuSO_4$ water solution 2.5 mM—which generated stress and injury in meristematic tissues (value determined experimentally, see Fig. 2, data point marked in black). All seedling incubations, as well as pre- or post-incubation with ATH (25 μM—final concentration), took place in climatic chamber at 25°C, in darkness, for 2 h. The plants were divided into the following experimental series: 1/water incubation (control); 2/ incubation with Cu^{2+} (Cu); 3/preincubation with the ATH-rich extract followed by incubation with Cu^{2+} (ATH > Cu); 4/incubation with Cu^{2+} together with the ATH-rich extract (ATH + Cu); 5/incubation with Cu^{2+} followed by postincubation with the ATH-rich extract (Cu > ATH).

Root meristems isolated from the control and treated seedlings were fixed (Ma et al. 1995) in Carnoy's solutions containing ethanol/glacial acetic acid (75/25; v/v) and kept at 4°C overnight. Root tips were then washed free of fixative with distilled water, hydrolyzed in 4 M HCl at room temperature for 60 min and stained with Schiff's reagent for 2 h at room temperature in the dark (Feulgen procedure). Squashed preparations of single root meristems were made on slides in 45% acetic acid. The slides were fixed according to the dry-ice procedure. The preparations were immersed in two changes of absolute ethanol and mounted in Histofluid (Medlab, Poland).

The MI was determined as the percentage of mitotic figures in 1,000 cells from the same root tip in at least three independent experiments (Glinska et al. 2007).

The scoring criteria for the MN have not been defined specifically for plant assays, but the criteria developed for MN experiments with mammalian cells can be adapted (Fenech 2000). A total of 4,000 cells isolated from four different root tips, for each sample were analysed.

Cytogenetic assay protocol for human lymphocytes

The tested substances were added to the medium after 48 h of culture. All cell incubations, as well as pre- or post-incubation with ATH (25 μM—final concentration) or 0.1 mM Cu^{2+} (value determined experimentally, see Fig. 3, data point marked in black), took place at 37°C, in darkness, for 1 h. The following experimental series were made: 1/RPMI 1640 medium incubation (control); 2/incubation with Cu^{2+} (Cu); 3/preincubation with the ATH-rich extract followed by incubation with Cu^{2+} (ATH > Cu); 4/incubation with Cu^{2+} together with the

ATH-rich extract (ATH + Cu); 5/incubation with Cu^{2+} followed by postincubation with ATH-rich extract (Cu > ATH).

The cells after incubations were prepared for MI and MN tests according to Błaszczyk and Skolimowski (2006). Cell were suspended in hypotonic solution (75 mM KCl, 3 min.) and fixed in MeOH-acetic acid (5:1, v/v). Air dried preparations were made and stained with 3% Giemsa stain.

The MI was determined as the percentage of mitotic figures in 1,000 cells from the same culture.

To perform MN cytochalasin-B (6 μg ml^{-1}) was added in the 44th h of culture to obtain binuclear cells. For each experimental variant 1,000 binucleared cells with well preserved cytoplasm were scored (Fenech 2000).

Statistical analyses

The data reported in this paper are presented as the mean ± SD. Statistical analysis was performed by variance analysis (ANOVA) using post-hock Duncan's multiple range test ($P < 0.05$).

Results

HPLC analysis of the extracts

The HPLC results of purified methanolic extract indicated the presence of cinnamic acid derivatives in the red cabbage leaves. It mainly contained hydroxycinnamic acids and anthocyanins which were predominated, constituting 84% of total phenolics (Table 1).

In the purified extract seven ATH-derivatives were clearly visible at 520 nm in HPLC chromatogram (Fig. 1d)—peak retention times: 9.05; 12.47; 25.02; 28.77; 29.6; 32.72 and 33.73. They were also visible at 280, 320 and 360 nm (Fig. 1a–c). The situation was similar with phenolic compounds defined as hydroxycinnamic acid derivatives—whose peaks were maximal at 320 nm (Fig. 1b) but their echo could also be seen at 280 and 360 nm (Fig. 1a, c).

Table 1 Content of major classes of phenolic compounds (PhC) in red cabbage purified extracts

PhC classes	(mg ml^{-1})	(mg g_{DM}^{-1})	%
Hydroxybenzoic acids	<0.001	–	–
Hydroxycinnamic acids	0.018	11	16
Flavonols	<0.001	–	–
Anthocyanins	0.099	58	84

Data determined by HPLC analysis

Cu^{2+} cytotoxicity

$CuSO_4$ concentrations optimal for further experiments were selected separately for each model system. The general assumption was that the cytotoxic factor (Cu^{2+}) should induce stress but could not be lethal for root meristems of *Vicia faba* (Fig. 2), or human lymphocytes (Fig. 3). Moreover, because of intentionally different cell models times of incubation were various: 2 h at 25°C for plant meristems and 1 h at 37°C in the case of lymphocyte cultures.

On the basis of the results presented in Fig. 2, 2.5 mM Cu^{2+} concentration was chosen as optimal for further experiments with root meristem cells of *Vicia* (data point marked in black). This solution caused ~50% inhibition of cell divisions (MI = 8.5) in comparison to the control, no-treated plant material (MI = 18.2). Higher and lower concentrations of Cu^{2+} were either too drastically or too slightly affecting *Vicia* root meristems during 2-h incubation.

In the MTT assay, the lymphocytes were incubated with different Cu^{2+} concentrations for 1 h. The survival curves (Fig. 3) show that Cu^{2+} was strongly cytotoxic for the lymphocyte cultures, after 1 h of incubation with Cu^{2+} 0.5 mM only 30% of the cells survived. The Cu^{2+} concentration causing 50% growth inhibition (IC_{50}) was 0.1 mM and this was chosen for further experiments (Fig. 3, data point marked in black).

Vicia faba cytogenetic tests

The highest mitotic activity was observed in the control untreated root tip meristems and those incubated with the 25 μM ATH extract (Fig. 4—grey stripes). Incubation with 2.5 mM Cu^{2+} caused 60% inhibition of cell divisions in *V. faba* root meristems. In all experimental variants where the meristematic tissues were treated with Cu^{2+} and the ATH extract (ATH > Cu; ATH + Cu; Cu > ATH) MI was higher then that of Cu^{2+} stressed only (Fig. 4—grey stripes).

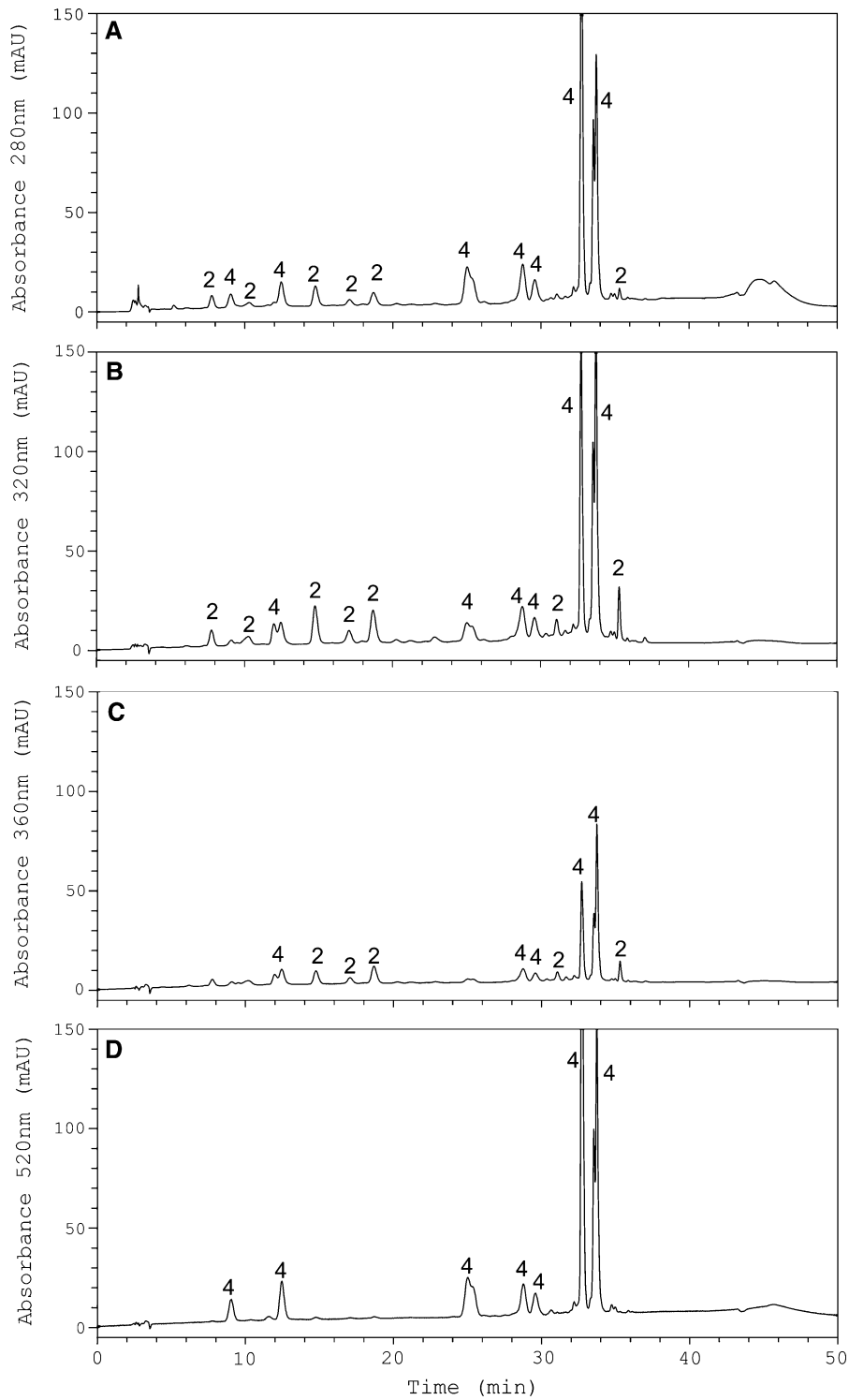

Fig. 1 HPLC profiles of phenolic compounds determined in the purified red cabbage extract. Each sample was analysed at four different wavelengths: **a** 280 nm; **b** 320 nm; **c** 360 nm; **d** 520 nm. 1—hydroxybenzoic acid derivatives; 2—hydroxycinnamic acid derivatives; 3—flavonols; 4—anthocyanins (ATH)

The appearance of MN showed a destructive effect of Cu^{2+} as well. The highest number of MN was observed in the cells treated with Cu^{2+} only (Fig. 5—grey stripes), which could suggest the clastogenic effect of Cu^{2+}. In the meristematic tissues incubated with the ATH extract the number of MN was

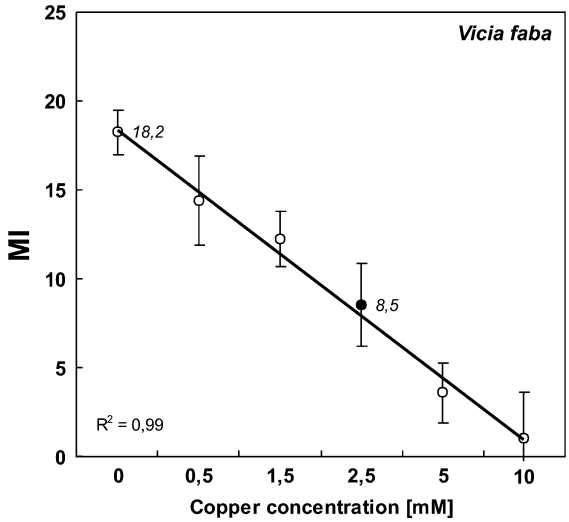

Fig. 2 Effect of different Cu^{2+} concentrations on mitotic activity of *V. faba* root meristem cells. The point marked in *black* represents the value of copper concentration which was chosen for further cytogenetic tests. MI—mitotic index (% of dividing cells). Means of 3–4 replicates ± SD

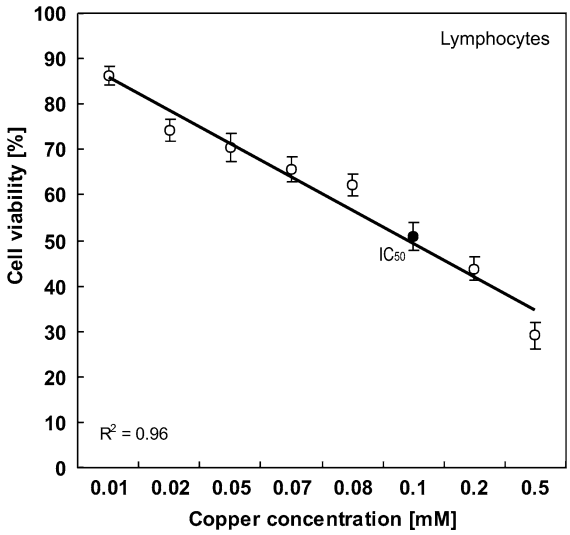

Fig. 3 Viability of human limphocyte cells treated with different Cu^{2+} concentrations. MTT test. The point marked in *black* represents the value of copper concentration which was chosen for further cytogenetic tests. Means of 3 replicates ± SD

comparable with that in the control untreated plants. On the other hand, the lowest frequency of MN occurrence was noted in the cells which were pre-incubated with Cu^{2+} and subsequently post-incubated with the ATH extract. The amount of MN in this experimental variant was 60% lower than that in the plants treated with 2.5 mM Cu^{2+}. In the meristematic cells pre-incubated with the ATH-rich extract and subsequently post-incubated with Cu^{2+} or incubated in the mixture of the tested substances micronuclei appeared with the frequency characteristic of the control (Fig. 5—grey stripes).

Human lymphocytes cytogenetic tests

The mitotic activity of the lymphocytes incubated with ATH was comparable to the control (Fig. 4—black stripes). The lowest MI was noted after Cu^{2+} treatment only, which suggests its high toxicity (Fig. 4—black stripes). In all three series with ATH and Cu^{2+} (ATH > Cu, ATH + Cu, Cu > ATH) mitotic was decreased in comparision to the control, however, higher than that after treatment with 0.1 mM Cu^{2+} only (Fig. 4). The highest MI values for the Cu^{2+} treated material were observed for the ATH > Cu series. (Fig. 4—black stripes).

The number of spontaneous MN observed in the control (8.75 ± 0.85) was typical of a healthy blood donor (Fig. 5—black stripes). In binuclear lymphocytes stressed with 0.1 mM Cu^{2+} a statistically significant increase in the mean number of micronuclei (39.7 ± 1.8) was observed in comparison to the control (Fig. 5—black stripes). After incubation with ATH only a slight increase in MN number was observed but it was not statistically significant (Fig. 5—black stripes). When the lymphocytes were incubated with both ATH and Cu^{2+} (ATH > Cu, ATH + Cu, Cu > ATH) an increase in the mean number of MN was observed in comparison to the control. However, if these results were compared to those obtained after Cu^{2+} stress only they were statistically lower at all variants. The lowest number of MN was observed in the treatment where 2.5 μM ATH and 0.1 mM Cu^{2+} applied together (ATH + Cu) (Fig. 5—black stripes).

Discussion

We aimed at finding a rich and cheap source of health beneficial substance or substances which can eliminate or limit negative effects caused by heavy metal environment contamination.

It is well known that red cabbage contains 23 ATHs which are all cyanidin derivatives mostly

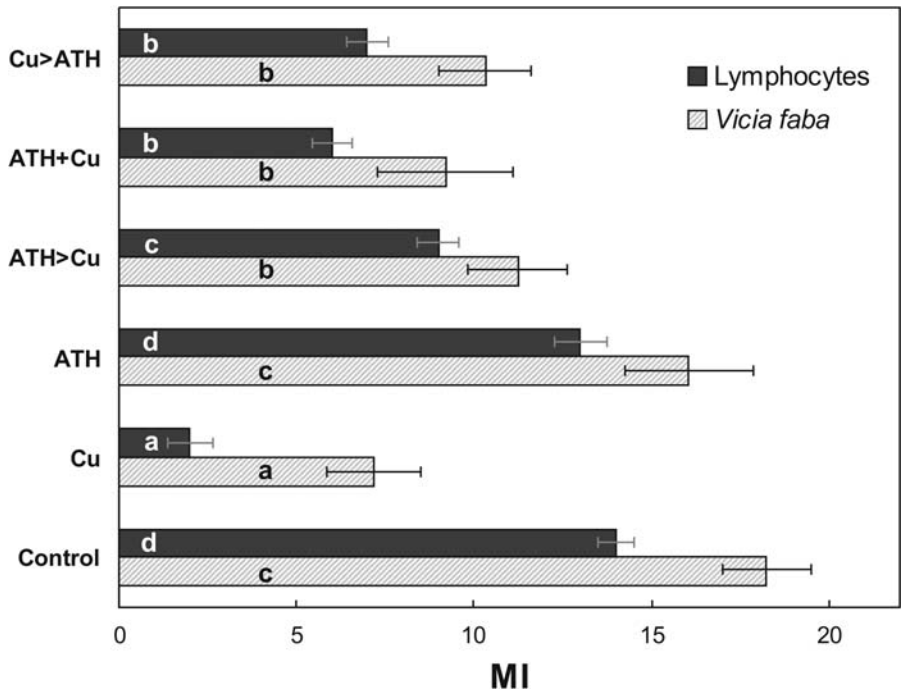

Fig. 4 Influence of the red cabbage ATH extract (ATH; 25 μM) on the mitotic activity of meristematic tissue of *V. faba* roots treated with 2.5 mM Cu^{2+} solution and of human lymphocytes treated with 0.1 mM Cu^{2+} solution. MI—mitotic index (% of dividing cells). Means of 4 repetitions ± SD, Data were subjected to analysis of variance using Duncan's multiple range test $P < 0.05$

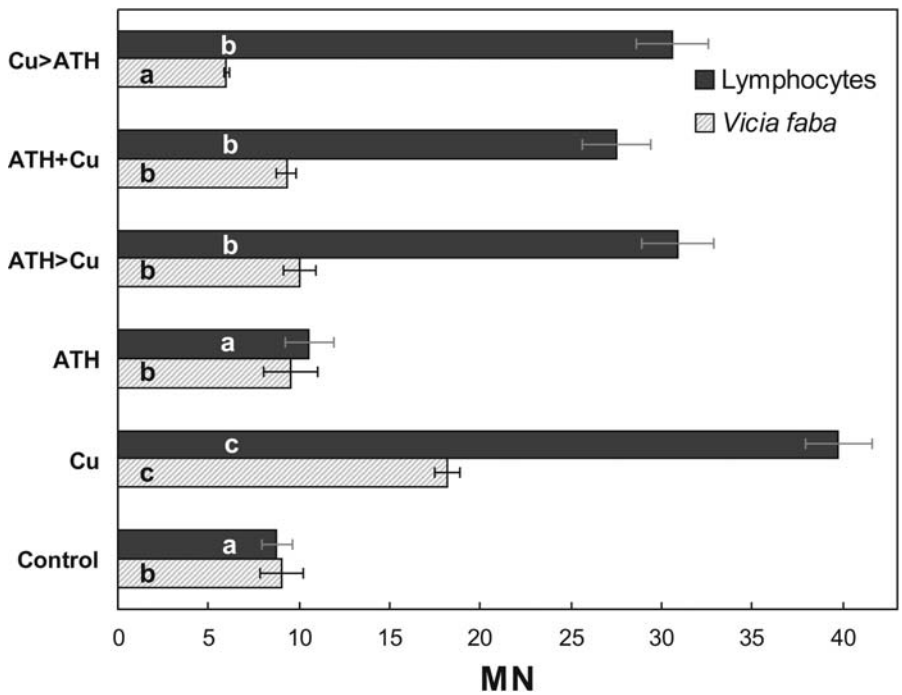

Fig. 5 Influence of the red cabbage ATH extract (ATH; 25 μM) on micronuclei (MN) appearance in meristematic tissue of *V. faba* roots treated with 2.5 mM Cu^{2+} solution and in human lymphocytes treated with 0.1 mM Cu^{2+} solution. Means of 4 repetitions ± SD, Data were subjected to analysis of variance using Duncan's multiple range test $P < 0.05$

cyanidin-3-diglucoside-5-glucoside with various acylated groups (e.g. sinapic, ferulic, caffeic, coumaric or malonic acids) connected to it. The paper by Wu and Prior (2005) is the only one which gives so detailed analysis of ATHs in red cabbage—this proves the complexity and difficulty of this identification research. So fractionating is laborious, too expensive and useless. It is well documented that

often a mixture of plant polyphenols present in nature, by their synergistic collaboration acts better that its individual components separately (Rice-Evans et al. 1997; Havsteen 2002). Moreover, experiments indicated that even after ATH extract purification there are always some small-molecular phenolics e.g. hydroxycinnamic acids, present (Table 1), as they can be separated from acylated ATHs. Howether, it is no problem if the investigated mixture exhibits demanded properties. Our earlier experiments showed that the mixture of ATH extracted from red cabbage possessed high antioxidant properties and suggested protective action of this extract against Pb, Cd and Cr toxicity (Glińska et al. 2007).

The presented data document cytotoxicity of high Cu^{2+} concentrations both for plant and animal tissues. Mitodepressive, turbogenic and cytotoxic effects of heavy metals were described for many plants (Wierzbicka 1999; Liu et al. 2003; Glińska et al. 2007) and animals (Bagchi et al. 2002; Fatur et al. 2002; Palus et al. 2003). Data from literature confirm that inhibitory effect of Cu^{2+} for example on root growth is due to the reduction of the cell division (Kahle 1993; Doncheva et al. 1996). Simultaneously, a beneficial effect of ATH from red cabbage extract on the mitotic activity of *Vicia* meristematic cells as well as inhibitory effect of ATH on Cu^{2+} cytotoxicity for human lymphocytes were observed. ATH mixture limited harmful effect of Cu^{2+} on plant and animal tissues. In all experimental series with ATH application MI was higher than that caused by Cu^{2+} treatment only. Pre-incubation with ATH gave the best results. Moreover, in all experimental series the number of MN was lower in comparison with the material stressed with Cu^{2+} only. ATH from natural sources limited genetic damage caused by clastrogenic effect of the metal.

Surprising good effect of ATH action was observed in *Vicia* root meristem cells treated with Cu^{2+} and post-incubated in the red cabbage extract. This may suggest that ATH not only significantly limits but heals cytological injury caused by Cu^{2+} as well.

Generally, ATH applied before and during Cu^{2+} stress protected cells against harmful heavy metal activity preventing genetic damage. The mechanisms of their action are not well understood. Experimental evidence suggests that polyphenols such as flavonoids including ATH can act through one or more mechanisms: 1/modulation of metabolic activation of mutagens (Enderharder and Tang 1997), 2/modulating antioxidant enzymes (Brandi 1992), 3/stimulating detoxification enzymes (Williams et al. 2002; Posmyk et al. 2009a), 4/they can ability to scavenge free radicals (Wang et al. 1997; Boveris et al. 2001; Kong et al. 2003; Tsuda et al. 2000; Glinska et al. 2007) limit secondary oxidative stress therefore inhibit lipoprotein and lipid peroxidation (Ramirez-Tortosa et al. 2001; Lazzé et al. 2003) 6/they are able to form strong ligand complexes with ions of metals, such as Fe, Cu and Mo (Aherne and O'Brian 2000; Ferguson 2001; Havsteen 2002). Their chelating properties lead to metal isolation, i.e. Mo sequestration in the epidermis of *Brassica* sp. (Hale et al. 2001).

The phenomenon of DNA structure stabilisation by ATH is widely discussed in literature. According to Lazzé et al. (2003), delphinidin effectively protects DNA against single strand breaks formation induced by *tert*-butyl-hydroperoxide. In Sarma and Sharma (1999) opinion, DNA stabilisation resulted from a direct ATH interaction with phosphate backbone. Probably cyanidin and DNA associate to form a complex and such a co-pigmentation protects both DNA and ATH from the damage caused by OH. Similarly Mas et al. (2000) investigated and finally confirmed abilities of different ATH-monoglucosides to stabilise DNA triple-helical complexes.

Moreover, ATH being antimutagens are probably able to protect DNA by reacting with electrophilic metabolites or by masking nucleophilic centres of DNA (De Flora 1998).

Therefore, it cannot be excluded that the lowered frequency of mitotic disturbances induced by Cu^{2+}, or even their disappearance as in *Vicia faba* root meristems treated with ATH may result from the antioxidant properties of the used extract and from the direct stabilisation of DNA by complexion with ATH.

Several well-known mutagenic risk factors such as heavy metal pollution are closely connected with modern civilisation. Therefore, there exists a need to reduce genotoxic effect of mutagenic and cancerogenic factors by regular intake of antigenotoxic agents. The best candidates appear to be natural diet components like ATH from red cabbage.

The results of the present research reveal the role of ATH-rich cabbage extract in the protection of the meristematic cells of *Vicia faba* roots as well as in

vitro cultured human lymphocytes, against heavy metal toxicity. In the plant model, the surprisingly good effect of ATH action was observed even after Cu^{2+} treatment. This may suggest that ATH not only significantly limits but heals cytological injury caused by Cu^{2+} as well.

Therefore, the beneficial proprieties of the red cabbage extract should be widely spread to popularise functional food and detoxifying diet reducing the risk of dangerous diseases resulting from heavy metal pollution of the environment. Moreover, it may become potentially attractive, inexpensive and readily available at a large scale, raw material for pharmaceutical, cosmetic and food industries.

In the other hamd, the positive effect of ATH on plant root meristems under stress suggest their phytobiostymulatoring role. Biostimulators could be used to stabilise agricultural and hydroponical systems and to counteract stress situations. Such a substances can be applied as sprays, fertilisers supplements or applicated direct into the seeds as presowing hydro- and osmopriming elements (Posmyk et al. 2008, 2009b; Glinska et al. 2007).

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