

Effect of anthocyanins on selected biochemical parameters in rats exposed to cadmium^{*}

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Cadmium is a dangerous occupational and environmental toxin. It accumulates in the human organism mainly in liver and kidneys. Cadmium half-life is about 10 years, so the symptoms of cadmium intoxication may occur several years after the exposure. Until now in treating intoxication with this metal chelating compounds have been used, burdened with numerous undesirable symptoms. In our investigations anthocyanins from *Aronia melanocarpa* were used to reduce the harmful results caused by cadmium. Administering anthocyanins with cadmium chloride resulted in a statistically significant decrease of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activity, concentration of bilirubin and urea in blood serum and decreased cadmium cumulation in liver and kidneys in relation to animals receiving cadmium chloride only.

Cadmium is one of the most dangerous occupational and environmental toxins. It is found in drinking water, atmospheric air and even in food. Products of vegetable origin are the main carrier of cadmium compounds in food (Kłos, 2001). Having been absorbed from the alimentary tract, cadmium forms durable combinations with the protein thionein, form-

ing metallothioneins which play an important role in further metabolism of this metal. Kidneys (mainly renal cortex) and liver are considered to be the most susceptible organs in the case of exposure to cadmium (Ryan *et al.*, 2000; Yamano *et al.*, 1999; Yiin *et al.*, 1999). The damaging effect of cadmium on the liver is manifested by an increase of aspartate

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Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; Hgb, haemoglobin; PTWI, provisional tolerable weekly intake; RBC, red blood cells.

aminotransferase (AST) and of the most specific marker of liver cell damage – alanine aminotransferase (ALT). Sauer *et al.* (1997) and Blasco & Puppo (1999) also observed an increase of the activity of these enzymes as a result of cadmium activity. Cadmium hepatotoxicity is probably effected in two ways: on the one hand by the occurrence of inflammatory state, on the other hand – by direct toxic action of cadmium on liver cells. So far in treating cadmium intoxications chelating compounds have been used, e.g. calcium disodium versenate, dimercaprol and meso-mercaptosuccinic acid (Shaikh *et al.*, 1999; Piotrowski *et al.*, 1995). In the recent years in scientific investigations attention has been drawn to the “health-promoting” activity of vegetable pigments, among them anthocyanins (Oszmiański, 2001; Hertog *et al.*, 1993). Due to the commonness of occurrence, anthocyanins are abundant components of human diet (fresh fruit, juices, wine). As yet, a toxic effect of anthocyanins on humans and animals has not been shown (Oszmiański, 2001). Anthocyanins demonstrate anti-inflammatory (Subarnas & Wagner, 2000) and antioxidative activity (Oszmiański, 2001; Subarnas & Wagner, 2000; Wolniak, 2002; Tsuda *et al.*, 1999; Wang, 2000), and they are able to chelate metal ions (Wawer, 2001). Anthocyanins increase the resistance of hepatocytes to oxidation, activate liver enzymes (aspartate aminotransferase, alanine aminotransferase and lactate dehydrogenase) and lower the reduced glutathione concentration in the liver.

The aim of the study was to evaluate the effect of anthocyanins from *Aronia melanocarpa* on the selected biochemical parameters in rats exposed to cadmium.

MATERIAL AND METHODS

Wistar rats (male) with mean mass 210 ± 15 g were kept in animal quarters with stable

temperature and humidity. The animals were divided into four groups, 10 in each of them. They received daily for 30 days with a stomach tube: group I (control) – 0.5 ml of drinking water; group II – aqueous solution of anthocyanins from *Aronia melanocarpa* (described below) 10 mg/kg of body mass; group III – in the form of aqueous solution, cadmium chloride ($\text{CdCl}_2 \times 2.5 \text{ H}_2\text{O}$) 4.0 $\mu\text{g}/\text{kg}$ of body mass; group IV – cadmium chloride in the dose as in group III and 4 h after administering cadmium chloride, anthocyanins as in group II. The aqueous solution of anthocyanins from *Aronia melanocarpa* (Agropharm, Poland) composed of: 3-O-cyanidin galactoside (64.5%), 3-O-cyanidin arabinoside (28.9%), 3-O-cyanidin xyloside (4.2%) and 3-O-cyanidin glucoside (2.4%). In the course of the experiment the animals were fed with standard feed stuff for small laboratory animals (Murigran) and they had free access to drinking water. After 30 days the animals were terminated in general narcosis and blood, liver and kidneys were collected for the determination of:

1. in blood, erythrocyte count, haemoglobin concentration, haematocrite value using SELAB system;
2. in blood serum, urea, creatinine, bilirubin levels and AST and ALT activity using a Kone-Pro biochemical analyzer and Bio-Merieux reagents;
3. in liver and kidneys, content of cadmium with atomic emission spectrometry with plasmonic excitation in a ICP PU 7000 apparatus (Unicam, Cambridge). Organs were mineralized in teflon containers in a closed system in a microwave oven MLS 1200 Mega (Milestone, Bergamo). Analytical curves were made from Baker Standards (Philipsburg, U.S.A.).

The results were analysed statistically with U Mann-Whitney non-parametric test using the Statistica program.

The investigations were carried out with the approval of the Local Ethics Committee No. Ł/BD/121.

RESULTS AND DISCUSSION

The effects of anthocyanins from *Aronia melanocarpa* on selected liver and renal function biochemical tests (ALT and AST activities, bilirubin, urea and creatinine serum concentration) and the content of cadmium in animals receiving cadmium chloride were presented in Table 1.

The mechanism of the harmful activity of cadmium is not fully explained. It seems that the toxicity of this metal on the one hand lies in a direct action of free cadmium ions not bound with metallothionein, while on the other hand, in forming (under the influence of cadmium) reactive radicals being able to change functions and structure of many systems and organs (Kowalczyk *et al.*, 2002;

Table 1. Effect of anthocyanins on selected biochemical parameters and content of cadmium in rats receiving cadmium chloride.

Conditions of experiments are described in Material and Methods.

| Parametr | Group | | | | Statistical significance cadmium vs cadmium + anthocyanins |
|-----------------------------------|------------|--------------|------------|---------------------------|--|
| | Controls | Anthocyanins | Cadmium | Cadmium + anthocyanins | |
| RBC $10^6/\mu\text{l}$ | 6.33±0.45 | 6.22±0.74 | 6.11±0.47 | 6.20±0.45 | NS |
| Hgb g/dl | 15.19±1.04 | 14.26±0.92 | 13.87±0.23 | 14.40±0.59 | NS |
| Hct % | 39.47±2.39 | 38.37±3.62 | 37.80±2.53 | 38.74±1.84 | NS |
| AST U/l | 109.00±15 | 111.50±35 | 142.00±33 | 113.00±20 | NS |
| ALT U/l | 43.00±5.5 | 32.50±4.4 | 76.00±6.4 | 35.00±5.2 | $P < 0.001$ |
| Bilirubin mg/dl | 0.43±0.1 | 0.60±0.1 | 0.60±0.05 | 0.45±0.05 | NS |
| Urea mg/dl | 31.00±4.3 | 37.00±5.17 | 50.40±3.12 | 37.90±4.1 | $P < 0.001$ |
| Creatinine mg/dl | 0.43±0.04 | 0.55±0.07 | 0.69±0.03 | 0.70±0.01 | NS |
| Cadmium in kidney $\mu\text{g/g}$ | 1.26±0.009 | 0.49±0.003 | 5.18±0.27 | 1.67±0.03 | $P < 0.001$ |
| Cadmium in liver $\mu\text{g/g}$ | 0.41±0.017 | 0.4±0.011 | 1.74±0.01 | 1.2±0.13 | $P < 0.001$ |

Administering cadmium chloride to rats resulted in a statistically significant decrease of haemoglobin concentration in blood in relation to the control group. Adding anthocyanins to cadmium chloride did not normalize the haemoglobin concentration, while it significantly decreased AST and ALT activity, concentration of bilirubin and urea in blood serum (the above parameters were increased after administering cadmium chloride alone). The anthocyanins also statistically significantly decreased the accumulation of cadmium in liver and kidneys.

Stojs *et al.*, 2001). As a result of exposure to cadmium there develops, among others, hypochromic anaemia. Several mechanisms leading to anaemia have been suggested. Cadmium may compete with iron, leading to the occurrence of anaemia due to iron deficiency, whereas on the other hand renal failure, developing under the influence of cadmium, results in erythropoietin deficiency which may cause normochromic and normocytic anaemia without a proportional increase of reticulocytes (Hiratsuka *et al.*, 1996; Mackova *et al.*, 1996). Chun *et al.* (2000) are of the opinion that cad-

mium ions significantly affect regulatory genes for erythropoietin, which may be the cause of inhibiting its expression. In our investigations a slight decrease of haematologic value was observed in the group of animals receiving cadmium, which may be connected with the short time of exposure (30 days) or with too small a dose of cadmium used in the experiment. According to FAO/WHO, provisional tolerable weekly intake (PTWI) for cadmium in man is 0.0067 mg/week per kg of body mass. Due to the quicker metabolism in rats, the dose of cadmium was increased four times. The application of anthocyanins resulted in a slight improvement of haematologic values.

It has been known for a long time that cadmium mainly accumulates in liver and kidneys because these organs contain most of metallothionein binding toxic metals (Choudhury *et al.*, 2001; Hollis *et al.*, 2001; Liu *et al.*, 1998; Yiin *et al.*, 1999). Of considerable interest is the decrease of cadmium accumulation in liver and kidneys in the animals receiving anthocyanins in addition to cadmium, observed in our investigation. It may testify to the possibility of anthocyanins chelating cadmium ions which in consequence may decrease the damages caused by this metal. Cadmium accumulation in liver and kidneys results in functional changes and then in interstitial fibrosis of these organs.

The anthocyanins used in our investigations demonstrate protective action against the damages of hepatocytes by cadmium, for they normalize the activities of liver enzymes (AST, ALT). A protective effect of anthocyanins on liver cells has also been observed by Tsuda *et al.* (1999).

Another sensitive indicator of liver cell damage is the concentration of total bilirubin in plasma. Novelli *et al.* (1998) reported a significant increase of total bilirubin concentration in rats exposed to cadmium. Sarkar *et al.* (1998) found that the increase of total bilirubin concentration in plasma after intoxication

with cadmium correlates with the oxidative damage of other organs resulting from oxidative stress.

In our investigations we observed a considerable increase of total bilirubin concentration in plasma of rats which were given cadmium chloride. Additional administration of anthocyanins resulted in a statistically significant lowering of the increase of total bilirubin concentration in plasma caused by cadmium treatment. The observed beneficial changes may result from the antioxidative activity of anthocyanins which was the subject of numerous scientific reports (Oszmiański, 2001; Subarnas & Wagner, 2000; Wolniak, 2002; Tsuda *et al.*, 1999; Wang, 2000).

As a result of cadmium activity one notices also renal tubule damage and then glomerular filtration impairment (Shibutani *et al.*, 2001). This may account for the increase of urea and creatinine concentration in the animals receiving cadmium chloride. The damaging effect of cadmium on kidneys has also been described by other authors (Jarup *et al.*, 2000; Jarup, 2002). Simultaneous administration of anthocyanins with cadmium chloride significantly decreases the concentration of urea in blood with no significant decrease of the creatinine value. The mechanism of the beneficial effect of anthocyanins on kidneys is undoubtedly heterogeneous. Anthocyanins decrease the inflammatory process (they inhibit cyclooxygenase-1 and -2) (Subarnas & Wagner, 2000), decontract blood vessels and improve microcirculation (Bertuglia, 1995).

In our investigations a decrease of concentration of oxidative stress biomarkers was observed in animals receiving cadmium chloride and anthocyanins in relation to those receiving only cadmium chloride (in preparation). Although the mechanism of the action of anthocyanins in human organism is still unexplained, their multidirectional activities account for the application of anthocyanins in many diseases.

CONCLUSIONS

1. Administering anthocyanins from *Aronia melanocarpa* with cadmium chloride decreases:

a) the activity of AST, ALT enzymes, bilirubin and urea concentration in blood serum;

b) cadmium concentration in liver and kidneys.

2. The use of anthocyanins in prophylaxis or therapy of people exposed to increased cadmium concentration requires carrying out investigations with the use of more numerous studied groups and evaluating the dependence of the effect upon the dose of anthocyanins.

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