

## EFFECT OF ARONIA MELANOCARPA FRUIT JUICE ON THE ACTIVITY OF ANTIOXIDANT ENZYMES IN A RAT MODEL OF AMIODARONE-INDUCED PNEUMOTOXICITY

**Stefka V. Valcheva-Kuzmanova,**  
**Galya Ts. Stavreva<sup>1</sup>,**  
**Violeta Y. Dancheva<sup>2</sup>,**  
**Ljudmil G. Terziev<sup>3</sup>,**  
**Veneta L. Shopova<sup>2</sup>**  
**Angelina M. Stoyanova<sup>4</sup>**

*Department of Preclinical and Clinical Pharmacology and Toxicology,*

*Medical University – Varna*

<sup>1</sup>*Department of Experimental and Clinical Pharmacology,*

*Medical University – Pleven*

<sup>2</sup>*Department of Disaster Medicine,*

*Medical University – Pleven*

<sup>3</sup>*Clinic of Allergology,*

*University Hospital – Pleven,*

<sup>4</sup>*Sector of Chemistry,*  
*University Hospital – Pleven,*  
*Bulgaria*

### Corresponding Author:

Stefka V. Valcheva-Kuzmanova  
Dept of Preclinical and Clinical Pharmacology and Toxicology,  
Medical University – Varna,  
55, Marin Drinov str.  
9002 Varna,  
Bulgaria  
e-mail: [stefkavk@yahoo.com](mailto:stefkavk@yahoo.com)

**Received:** August 18, 2012

**Revision received:** October 20, 2012

**Accepted:** November 27, 2012

### Summary

The effect of *Aronia melanocarpa* fruit juice (AMFJ) on the activity of antioxidant enzymes in a model of amiodarone (AD)-induced pneumotoxicity in rats was studied. AD was instilled intratracheally on days 0 and 2 (6.25 mg/kg as a 3.125 mg/mL water solution). AMFJ (5 mL/kg and 10 mL/kg) was given orally from day 1 to days 2, 4 and 9. The activities of catalase (CAT), glutathione peroxidase (GPx) and superoxide dismutase (SOD) in lung tissue were measured on days 3, 5 and 10, respectively. AD decreased significantly CAT activity on days 3, 5 and 10. It caused a decrease of GPx activity which was significant on day 3. It decreased SOD activity but not significantly. AMFJ antagonized the effects of AD to such an extent that the enzyme activities at all time points did not differ significantly from the control values. The effect of AMFJ is probably due to its polyphenolic ingredients which serve as powerful radical scavengers. AMFJ probably decreased of the oxidative damage of cells by AD-induced overproduction of reactive oxygen species thus preserving the capacity of cells to produce antioxidant enzymes which, in turn, could further reduce oxidative stress.

**Key words:** *Aronia melanocarpa* fruit juice, amiodarone, pneumotoxicity, antioxidant enzymes

### Introduction

Amiodarone is a benzofuran derivative with highly effective class III antidysrhythmic activity. However, its use is associated with many side effects involving many different organ systems. The most serious side effect of amiodarone is pulmonary fibrosis. It has been postulated that the cause of AD-induced pneumotoxicity is complex and multifactorial, possibly involving several mechanisms [1]. Free radical formation, direct cytotoxicity, development of lysosomal phospholipidosis and membrane destabilization are the documented possible cellular mechanisms of toxicity, but it is known that the predominant molecular mechanism of AD-induced cell death is the oxidative damage [2]. The oxidative stress is normally due either to increased production of reactive oxygen species (ROS) or decreased antioxidant capacity of cells. Oxidative stress induced by AD has been demonstrated by the

increased concentration of oxidized glutathione formation [3, 4] as well as by the increased superoxide formation [3]. The literature data concerning the effects of AD on the activity of antioxidant enzymes in the lung are controversial [4-6].

Since it is not possible to prevent the development of oxidative stress itself, it would be rational to increase the cellular levels of antioxidants in order to reduce AD-induced lung damage. Consequently, both catalytic and scavenger antioxidants have been shown to attenuate AD-induced lung injury and fibrosis in animals [7].

*Aronia melanocarpa* [Michx.] Elliot (black chokeberry) fruits are extremely rich in phenolic compounds: procyanidins, flavonoids (mainly from the subclass of anthocyanins) and phenolic acids (chlorogenic and neochlorogenic) [8]. A series of studies has investigated the antioxidant properties of *Aronia* juice, *Aronia* extract or its phenolic constituents using different well established assays [8-14]. Fresh *Aronia* berries possess the highest antioxidant capacity among berries and other fruits investigated so far as measured with oxygen radical absorbance capacity [9, 11].

Up to now, there are no literature data on the effect of *Aronia melanocarpa* in AD-induced lung damage.

The aim of the present study was to investigate the effect of *Aronia melanocarpa* fruit juice (AMFJ) on the activity of antioxidant enzymes (catalase, glutathione peroxidase and superoxide dismutase) in a rat model of AD-induced pneumotoxicity.

## Materials and Methods

### Experimental substances

Amiodarone hydrochloride (AD) and all other chemicals and reagents were of analytical grade and were purchased from Sigma-Aldrich Company (Germany).

AMFJ was produced from *Aronia melanocarpa* Elliot fruits grown in the Balkan Mountains, Bulgaria. They were handpicked in September, crushed and squeezed. The juice was filtered, pasteurized at 80°C for 10 min and stored at 0°C till the experiment. The contents of phenolic substances in 100 mL AMFJ were: total phenolics, 709.3±28.1 mg as gallic acid equivalents, determined spectrophotometrically according to the Folin-Ciocalteu procedure [15]; total flavonoids, 189.4±8.6 mg as catechin

equivalents, measured by a colorimetric assay developed by Zhishen et al. [16]; total anthocyanins, 106.8±6.2 mg as cyanidin-3-glucoside equivalents, determined by a pH-differential spectrophotometry at pH 1.0 and pH 4.5 [17]; quercetin, 11.8 mg, measured by a high-performance liquid chromatography method [18]. The values were the mean of duplicate determinations of three samples.

### Animals and experimental treatments

The study was carried out on 72 male Wistar rats (weight 220-250 g, age 4 months). The animals were obtained from the Research and Laboratory Animal Breeding Center of Slivnitsa (Bulgaria) and were housed in the university animal quarters for 1 month at a temperature of 22±2°C and humidity of 50±10%, given normal pelleted diet and water ad libitum.

All procedures concerning animal treatment and experimentation were conducted in compliance with the national laws and policies, in conformity with the international guidelines (EEC Council Directive 86/609, IL 358, 1, December 12, 1987). The experiment was approved by the Ethic's Committee of Medical University – Pleven.

The animals were divided into four groups of 18 rats. Each group was subdivided into 3 subgroups of 6 rats. The subgroups were respectively sacrificed on days 3, 5 and 10 under thiopental anesthesia (50 mg/kg) by exsanguination through cutting v. renalis.

Group 1 (Control) received two intratracheal (i.t.) instillations of sterile distilled water (2 mL/kg) on days 0 and 2, and the three subgroups received distilled water (10 mL/kg) orally through an orogastric cannula from day 1 to days 2, 4 and 9, respectively. Group 2 (AD) received two i.t. instillations of AD (6.25 mg/kg, as a 3.125 mg/mL water solution) on days 0 and 2 [19] and the subgroups received distilled water (10 mL/kg) orally through an orogastric cannula from day 1 to days 2, 4 and 9, respectively. Group 3 (AD + AMFJ5) was treated with AD i.t. on days 0 and 2, and from day 1 to days 2, 4 and 9 the respective subgroups received AMFJ orally at a dose of 5 mL/kg diluted with distilled water to a total volume of 10 mL/kg. Group 4 (AD + AMFJ10) was treated with AD i.t. on days 0 and 2, and from day 1 to days 2, 4 and 9 the respective subgroups received AMFJ orally at a dose of 10 mL/kg.

AD was dissolved in distilled water at 60°C and allowed to cool to room temperature before

the i.t. instillation.

### Biochemical assays of lung homogenate

Lung homogenate was obtained from the right lung. The tissue was homogenized with KCl (1.15 %) in 1:10 ratio. The homogenate was centrifuged (9000 x g, 30 min), and the supernatant was stored on ice. Catalase (CAT) activity in mcat/g tissue was measured by the method of Koroljuk et al. [20]. Glutathione peroxidase (GPx) in U/g tissue was measured by the method of Berchneider, modified by Pereslegina [21]. Superoxide dismutase (SOD) activity in U/g tissue was measured by the method of Maral et al. [22].

### Statistical analysis

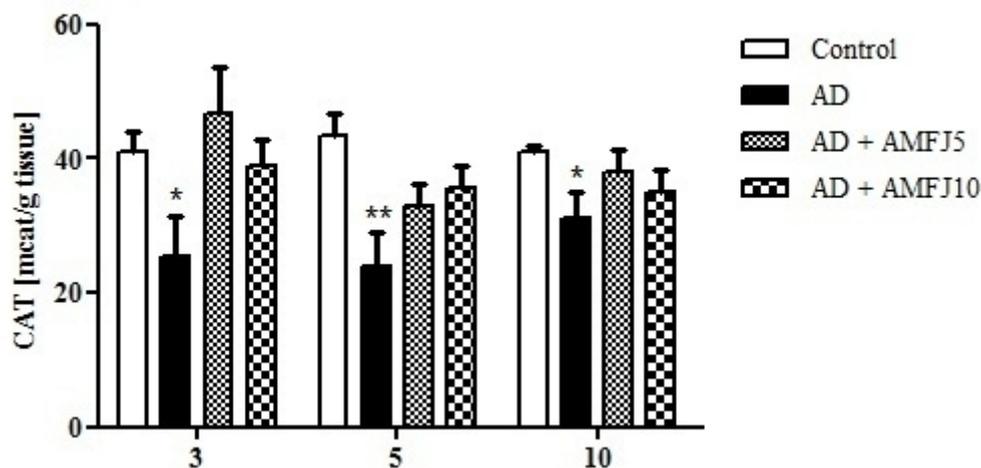
Results are presented as mean  $\pm$  S.E.M. The data were tested by one-way ANOVA, followed by Dunnett's multiple comparison post test. A level of  $p < 0.05$  was considered significant. All analyses were performed using GraphPad Prism

statistical software.

## Results

### CAT activity

AD instillation resulted in a significant decrease of CAT activity on day 3 to a level that was 62 % of the control value ( $p < 0.05$  vs control), on day 5 to a level that was 55 % of the control value ( $p < 0.01$  vs control) and on day 10 to a level that was 76 % of the control value ( $p < 0.05$  vs control) (Figure 1). Thus, the inhibition of enzyme activity was greatest on day 5. AMFJ prevented AD-induced reduction of CAT activity. In rats from AD + AMFJ5 group, CAT activity did not differ significantly from the control level and was 114 %, 77 % and 93 % of the control value on days 3, 5 and 10, respectively. In rats from AD + AMFJ10 group, CAT activity was not significantly different from the control one and was 95 %, 82 % and 85 %, of the control level on days 3, 5 and 10, respectively (Figure 1).

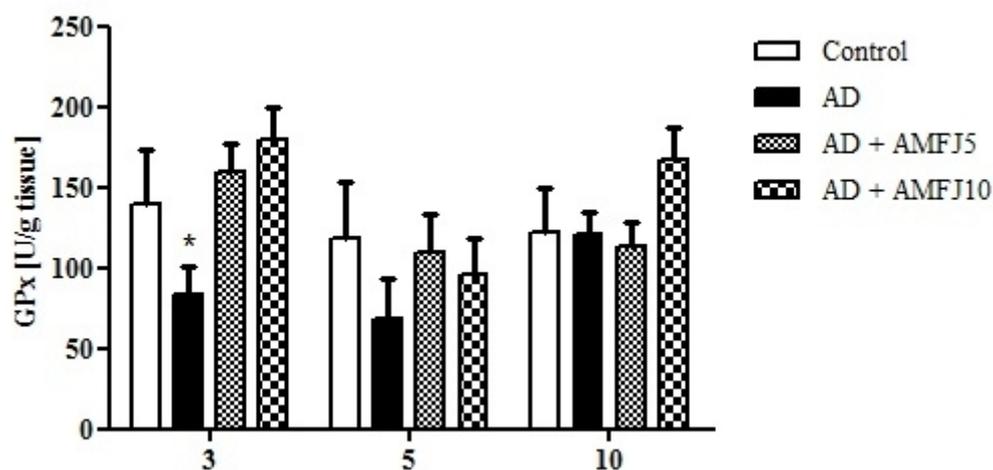


**Figure 1.** Effect of *Aronia melanocarpa* fruit juice (AMFJ) at doses of 5 and 10 mL/kg on catalase (CAT) activity in rat lung tissue 3, 5 and 10 days after intratracheal instillation of amiodarone (AD). \* $p < 0.05$  vs Control; \*\* $p < 0.01$  vs Control

### GPx activity

Gpx activity is presented in Figure 2. In AD group, GPx activity was reduced to a level that was 60% of the control value ( $p < 0.05$  vs control) on day 3, 57% of the control on day 5 and 99% of the control on day 10. AMFJ antagonized that effect of AD. In AMFJ-treated animals, GPx activity did not differ significantly from the control one at all time points. Thus, in rats from

AD + AMFJ5 group, GPx activity was 114%, 92% and 93% of the control value on days 3, 5 and 10, respectively. In rats from AD + AMFJ10 group, GPx activity was 128%, 81% and 137% of the control activity on days 3, 5 and 10, respectively (Figure 2).

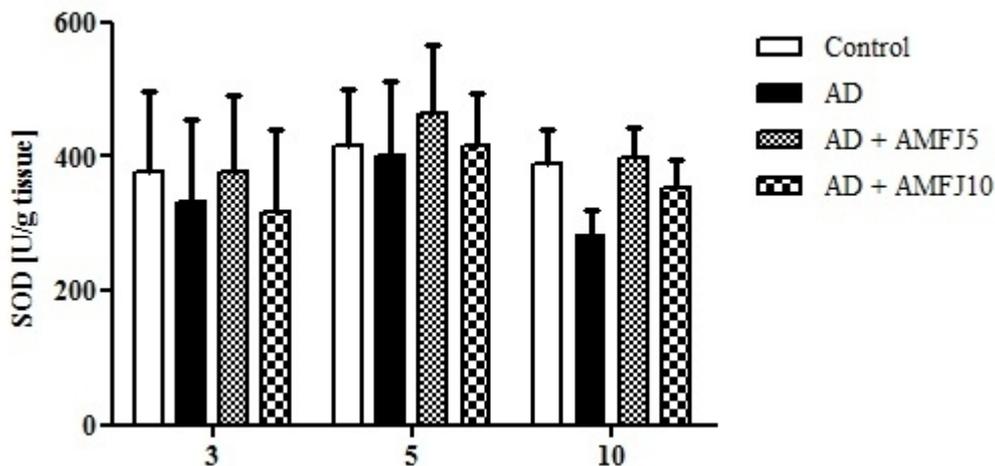


**Figure 2.** Effect of Aronia melanocarpa fruit juice (AMFJ) at doses of 5 and 10 mL/kg on glutathione peroxidase (GPx) activity in rat lung tissue 3, 5 and 10 days after intratracheal instillation of amiodarone (AD). \* $p < 0.05$  vs Control

**SOD activity**

As is obvious from Figure 3, AD had no significant effect on SOD activity. The enzyme activity of rats from AD group was 88% of the control value on day 3 and 96% of the control value on day 5. The effect of AD was greatest on day 10 when the enzyme activity was reduced to 73% of the control level but the effect was not statistically significant. At most time points and rat subgroups AMFJ antagonized the AD-induced tendency for reduction of SOD activity.

Thus, the enzyme activity in rats from AD + AMFJ5 group did not differ significantly from the control level and was 100% of the control value on day 3, 111% of the control on day 5 and 102% of the control on day 10. The enzyme activity in rats from AD + AMFJ10 group was also not significantly different from the control one and was 84% of the control value on day 3, 100% of the control on day 5 and 91% of the control on day 10 (Figure 3).



**Figure 3.** Effect of Aronia melanocarpa fruit juice (AMFJ) at doses of 5 and 10 mL/kg on superoxide dismutase (SOD) activity in rat lung tissue 3, 5 and 10 days after intratracheal instillation of amiodarone (AD)

## Discussion

There are numerous pathologic similarities between human and rodent lungs, and as such, rodent model presents an excellent tool to investigate pathologic changes *in vivo* [23]. The present study investigated the effect of AMFJ on the activity of antioxidant enzymes in a rat model of AD-induced pneumotoxicity.

CAT, SOD and GPx are antioxidant enzyme families that may act co-operatively in protecting cells against oxidative stress. CAT is a heme-containing enzyme found in peroxisomes that catalyzes the dismutation of hydrogen peroxide ( $H_2O_2$ ) to water and molecular oxygen [24]. GPx is a selenium-containing enzyme that catalyzes the oxidation of glutathione to glutathione disulfide, the ratio of which can be used as an indicator of oxidative stress [25], and is involved in the metabolism of lipid hydroperoxides and  $H_2O_2$  [26]. SOD catalyzes the metabolism of superoxide anions to hydrogen peroxide and molecular oxygen using a variety of cofactors such as copper, zinc, manganese, and iron [24].

Amiodarone-induced inflammation involves the recruitment of specific inflammatory cells such as polymorphonuclear leukocytes, lymphocytes, plasma cells and eosinophils [27, 28]. Inflammatory cells are capable of releasing ROS [29]. ROS-induced damage includes lipid peroxidation, protein polymerization and DNA strand breakage.

In this study, AD decreased the activity of CAT and GPx and had insignificant effect on the activity of SOD. These findings on the effect of AD on CAT and GPx activities are in accordance with the findings of other authors [6] who investigated the effect of AD administered intraperitoneally to rats. However, there are studies of other researchers demonstrating that AD had no effect on the activity of SOD, CAT and GPx in rat liver mitochondria [5] or even increased the activity of SOD in hamsters after intratracheal administration [4].

In the present study, the inhibition of antioxidative protective enzymatic system by AD probably resulted in cumulation of ROS and subsequent oxidative stress-induced pulmonary toxicity which was demonstrated by pathological changes in bronchoalveolar lavage fluid and lung tissue (unpublished data).

The results from the present study show that AMFJ antagonized the effect of AD antioxidant enzymes CAT, GPx and SOD and preserved the

enzyme activities to levels that were not significantly different from the control ones. The effect of AMFJ was most pronounced on the activity of CAT which was inhibited to a greatest extent by AD. These findings are in accordance with finding of other authors. Kowalczyk et al. [30] established that aronia anthocyanins increased CAT activity in mice intoxicated with sulphide-2-chloroethyl-3-chloropropyl. The study of Kedzierska et al. [31] demonstrated that an extract from *Aronia melanocarpa* berries reduced the changes in activities of different antioxidative enzymes (GPx, SOD, and CAT) in platelets treated with  $H_2O_2$ . Juice pretreatment resulted in an increase in the activity of CAT, GPx and glutathione reductase in rats challenged with N-nitrosodiethylamine [32]. *Aronia melanocarpa* extract increased the activity of SOD and GPx in patients with metabolic syndrome [33].

The preservation of the activity of antioxidant enzymes by AMFJ might contribute to its protective effect in AD-induced pneumotoxicity. Probably due to its radical scavenging activity, demonstrated by different well established assays [8-14], AMFJ reduced the damaging effect of ROS on cellular functions thus preserving the capacity of the cells to produce antioxidant enzymes. These enzymes, in turn, could further increase the protection of lung cells against oxidative damage.

## Conclusions

AD significantly reduced the activities of CAT and GPx and displayed a tendency to decrease SOD activity in a rat model of AD-induced pneumotoxicity. AMFJ antagonized these effects of AD. AMFJ probably decreased of the ROS-induced oxidative damage to cells thus preserving their capacity to produce antioxidant enzymes which, in turn, could further reduce oxidative stress.

## References

1. Reasor MJ, Kacew S. An evaluation of possible mechanisms underlying amiodarone-induced pulmonary toxicity. *Proc Soc Exp Biol Med.* 1996;212(4):297-304.
2. Golli-Bennour EE, Bouslimi A, Zouaoui O, Achour A, Bacha H. Cytotoxicity effects of

- amiodarone on cultured cells. *Exp Toxicol Pathol.* 2012;64(5):425-30.
3. Kennedy TP, Gordon GB, Paky A, McShane A, Adkinson NF Jr, Peters SP, et al. Amiodarone causes acute oxidant lung injury in ventilated and perfused rabbit lungs. *J Cardiovasc Pharmacol.* 1988;12(1):23-36.
  4. Leeder RG, Brien JF, Massey TE. Investigation of the role of oxidative stress in amiodarone-induced pulmonary toxicity in the hamster. *Can J Physiol Pharmacol.* 1994;72(6):613-21.
  5. Ribeiro SM, Campello AP, Nascimento AJ, Kluppel ML. Effect of amiodarone (AMD) on the antioxidant enzymes, lipid peroxidation and mitochondrial metabolism. *Cell Biochem Funct.* 1997;15(3):145-52.
  6. Al-Shammari BM, Khalifa A, Mohamed M. Submitted in partial fulfillment of the requirements for the master's degree in toxicology. 2009. Available from: <http://www.hdl.handle.net/123456789/19311>
  7. Brian JD. Antioxidants as potential therapeutics for lung fibrosis. *Antioxid Redox Signal.* 2008;10(2):355-70.
  8. Oszmianski J, Wojdylo A. Aronia melanocarpa phenolics and their antioxidant activity. *Eur Food Res Technol.* 2005;221:809-13.
  9. Zheng W, Wang SY. Oxygen radical absorbing capacity of phenolics in blueberries, cranberries, chokeberries, and lingonberries. *J Agric Food Chem.* 2003;51(2):502-9.
  10. Bermudez-Soto MJ, Tomas-Barberan FA. Evaluation of commercial red fruit juice concentrates as ingredients for antioxidant functional juices. *Eur Food Res Technol.* 2004;219:133-41.
  11. Wu XL, Gu LW, Prior RL, McKay S. Characterization of anthocyanins and proanthocyanidins in some cultivars of Ribes, Aronia, and Sambucus and their antioxidant capacity. *J Agric Food Chem.* 2004;52:7846-56.
  12. Valcheva-Kuzmanova S, Gadjeva V, Ivanova D, Belcheva A. Antioxidant activity of Aronia melanocarpa fruit juice in vitro. *Acta Aliment.* 2007;36:425-8.
  13. Jakobek L, Seruga M, Krivak P. The influence of interactions among phenolic compounds on the antiradical activity of chokeberries (*Aronia melanocarpa*). *Int J Food Sci Nutr.* 2011;62(4):345-52.
  14. Valcheva-Kuzmanova S, Blagović B, Valić S. Electron spin resonance measurement of radical scavenging activity of *Aronia melanocarpa* fruit juice. *Phcog Mag.* 2012;8(30):171-4.
  15. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. *Am J Emol Viticult.* 1965;16:144-58.
  16. Zhishen J, Mengcheng T, Jianming W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chem.* 1999;64:555-9.
  17. Guisti MM, Rodrigues-Saona LE, Wrolstad RE. Spectral characteristics, molar absorptivity and color of pelargonidin derivatives. *J Agric Food Chem.* 1999;47:4631-7.
  18. Hertog MGL, Hollman PCH, Vanema DP. Optimization of a quantitative HPLC determination of potentially anticarcinogenic flavonoids in vegetables and fruits. *J Agric Food Chem.* 1992;40:1591-8.
  19. Taylor MD, Van Dyke K, Bowman LL, Miles PR, Hubbs AF, Shannon R et al. A characterization of amiodarone-induced pulmonary toxicity in F344 rats and identification of surfactant protein-D as a potential biomarker for the development of the toxicity. *Toxicol Appl Pharmacol.* 2000;167(3):182-90.
  20. Koroljuk MA, Ivanova LI, Maiorova IG, Tokarev VE. A method of determining catalase activity. *Lab Delo.* 1988;1:16-9. [In Russian]
  21. Vlasova SN, Shabunina EI, Pereslegina IA. Activity of glutathione-dependent enzymes of erythrocytes in chronic liver disease in children. *Lab Delo.* 1990;8:19-22. [In Russian]
  22. Maral I, Puget K, Michelson H. Comparative study of SOD, catalase and GSH-Px levels in erythrocytes of different animals. *Biochem Biophys Res Commun.* 1977;77:1532-5.
  23. Moeller A, Rodriguez-Lecompte JC, Wang L, Gauldie J, et al. Models of pulmonary fibrosis. *Drug Discov Today Dis Models.* 2006;3:243-49.
  24. Harris ED. Regulation of antioxidant enzymes. *FASEBJ.* 1992;6:2675-83.
  25. Carey HV, Rhoads CA, Aw TY. Hibernation induces glutathione redox imbalance in ground squirrel intestine. *J Comp Physiol B.* 2003;173:269-76.
  26. Arthur JR. The glutathione peroxidases. *Cell Mol Life Sci.* 2000;57:1825-35.
  27. Martin WJ, Rosenow EC. Amiodarone pulmonary toxicity: Recognition and pathogenesis (Part 1). *Chest.* 1988;93:1067-75.
  28. Stavreva GT, Shopova VL, Dancheva VY, Stoyanova AM, Terziev LG. Effect of 21-aminosteroid U-74389G on markers for pneumotoxicity in rat bronchoalveolar lavage fluid after intratracheal amiodarone administration. *J Biomed Clin Res.* 2008;1(1):30-40.
  29. Laskin DL, Pendino KJ. Macrophages and inflammatory mediators in tissue injury. *Annu Rev Pharmacol Toxicol.* 1995;35:655-77.
  30. Kowalczyk E, Charyk K, Fijałkowski P, Niedworok J, Błaszczak J, Kowalski J. Protective Influence of natural anthocyanins of *Aronia melanocarpa* on selected parameters of antioxidative status in experimental intoxication with sulphide-2-chloroethyl-3-chloropropyl. *Pol J Environ Stud.* 2004;13(3):339-41.
  31. Kedzierska M, Olas B, Wachowicz B, Stochmal A,

- Oleszek W, Erler J. Changes of platelet antioxidative enzymes during oxidative stress: the protective effect of polyphenol-rich extract from berries of *Aronia melanocarpa* and grape seeds. *Platelets*. 2011;22(5):385-9.
32. Kujawska M, Ignatowicz E, Ewertowska M, Oszmiański J, Jodynis-Liebert J. Protective effect of chokeberry on chemical-induced oxidative stress in rat. *Hum Exp Toxicol*. 2011;30(3):199-208.
33. Broncel M, Koziróg M, Duchnowicz P, Koter-Michalak M, Sikora J, Chojnowska-Jezińska J. *Aronia melanocarpa* extract reduces blood pressure, serum endothelin, lipid, and oxidative stress marker levels in patients with metabolic syndrome. *Med Sci Monit*. 2010;16(1):CR28-34.