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EFFECT OF ANTHOCYANINS FROM *ARONIA MELANOCARPA* ON THE EXERCISE-INDUCED OXIDATIVE STRESS IN RAT TISSUES

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Abstract. We investigated the effect of the extract from fruits of *Aronia melanocarpa* (AM), containing the anthocyanin antioxidants on the lipid peroxidation index (TBARS) and the content of reduced glutathione (GSH) in rat tissues at rest and after exercising until exhaustion on a treadmill. For four consecutive days the animals were given AM through a gastric probe at 0.7 mg·kg^{-1} body mass (related to the content of the active substance). Control rats received 0.9% NaCl solution. Samples of the liver (L), heart (H), and white (WG) and red (RG) portions of the *gastrocnemius* muscle were collected from the animals at rest and immediately after the exercise. No effect of AM on TBARS was detected in the resting animals. The exercise, however, led to the significant elevation of the value of this index ($P<0.05$) in each of the tested tissues obtained from the control animals, and in the liver and heart of the AM-fed rats. The TBARS content in RG was significantly lower ($P<0.05$) in the latter compared to the former group of the animals. After administration of AM in rest, the GSH content tended to decrease in the examined tissues. Following the exercise, the significant reduction ($P<0.05$) in the GSH content was detected in all the tested tissues obtained from the control group. In contrast, no effect of the exercise on the GSH content was found in the AM-fed rats. After exercising, the higher GSH content ($P<0.05$) in the RG and H as well as the tendency to higher GSH content in WG and L were detected in rats given AM as compared to the control animals. The obtained results suggest that administration of AM markedly mitigates the exercise-induced reduction in the GSH content and elevation of TBARS in the tissues of the investigated animals.

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Key words: Antioxidants – Anthocyanins – Exercise – Glutathione – TBARS

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Introduction

Numerous reports from the literature have demonstrated antioxidative activities of anthocyanins and other flavonoids in the *in vitro* systems [7,26,30] as well as their presumably prophylactic effect in diseases the pathogenesis of which is related to the activity of free oxygen radicals [13,15,20]. The antioxidative function of anthocyanins was shown to be associated with scavenging of the hydroxyl radical and the superoxide anion [30]. The antioxidative effect *in vivo* of anthocyanins obtained from the *Aronia melanocarpa* fruits was first demonstrated by us in an earlier study [9]. The fruits contain anthocyanins from the flavonoid group, predominantly cyanidin 3-0-galactoside and cyanidin 3-0-arabinoside [25]. Administration of the A*ronia melanocarpa* extract to animals before the exhaustive exercise inhibited the increase in the lipid peroxidation index (content of thiobarbituric acid reactive substances; TBARS) in the red portion of the *gastrocnemius* muscle [9]. However, no effect of AM on lipid peroxidation could be demonstrated in other tissues studied (i.e., liver, heart, and white portion of *gastrocnemius*). The limited effect of the preparation could result from the relatively low dose used (approx. 0.36 $mg \cdot kg^{-1}$ body mass related to the content of the active substance), similar to the average flavonoids intake by humans in the diet which, according to Hertog *et al.* [13], approximates 25.9 mg per person per day (i.e., on the average 0.37 mg per kg b.m.). In fact, no harmful health effects of the AM fruits or its products have been reported in the literature. Notably, only few antioxidants can inhibit *in vivo* the exercise-induced increase in lipid peroxidation (see in 5,6). These results prompted us to reinvestigate the effect of AM administered at dose twice as high as that used in our previous study. For this purpose, concentration of TBARS and the reduced glutathione content (GSH) in the liver, heart, and red and white portions of the *gastrocnemius* muscle were chosen as indices of peroxidation processes in tissues.

Material and Methods

The investigation was conducted on 60 Wistar male rats weighing approx. 285±9 g each. The animals were fed *ad libitum* a standard diet for rodents (Murigram). All the animals were divided at random into four groups: control-rest (CR), control-exercise (CE), AM-fed-rest (AR) and AM-fed-exercise (AE). The animals from the AR and AE groups were given for four consecutive days 0.2 ml per day the 1:10 water solution of the AM fruits' extract (Agropharm, Tuszyn, Poland) through the gastric probe; the last dose was administered 60 min. before

the beginning of the experiment. According to the producer, concentration of the anthocyanin pigments in the extract equals to 989-1025 mg per 100 ml. Thus, the dose of the anthocyanins fed to the rats equalled to approx. 0.7 $mg \cdot kg^{-1}$ body mass. Rats from the CR and CE groups were given 0.9% NaCl solution. The animals were adapted to exercising on a treadmill set with electric stimulation for five consecutive days, five min. per day. After that, and after two additional days of rest, the animals from the CE and AE groups ran until exhaustion on the treadmill at the average speed of the track of 28 $m \cdot min^{-1}$ (27-29 $m \cdot min^{-1}$). The exhaustion was described as a state in which the animals after placing them three times on the treadmill were unable to run longer than for 10 s. Immediately after the exercise, the rats were killed by cervical dislocation and tissue samples were collected from the liver (L), heart (H) and *gastrocnemius* muscle. The muscle was divided into a red portion (RG) containing primarily the oxidative-glycolytic fibres and a white portion (WG) composed largely from the glycolytic fibres [2]. The collected tissues were immediately frozen at –70°C. Content of TBARS in the tissues was estimated spectrophotometrically as described by Okhawa *et al.* [24] and the reduced form of glutathione (GSH) was quantitated based on the concentration of the non-protein SH groups according to Sedlak and Lindsay [28]. The tissue protein content was estimated according to Lowry *et al.* [23].

After verification of a normal distribution, the results were analysed by the two-way analysis of variance (exercise x administration of AM in each tissue and then type of muscle x administration of AM at rest and after the exercise). Statistical significance of the differences between means was estimated post hoc using the Newman-Keuls test. The difference in time of the running until exhaustion was analysed using the Student t test. The differences between mean values were regarded as significant at $P<0.05$.

Results

Administration of AM did not significantly affect the time of the run until exhaustion (124.4±8.0 vs. 130.0±7.1 min.). The analysis of variance did not reveal any effect of the administration of AM on the concentration of TBARS in the investigated tissues at rest. However, as indicated in Fig. 1, after the exercise the index increased significantly in the liver and heart of both the AM-treated and control animals. In the red and white portions of the *gastrocnemius* muscle a significant post-exercise elevation of TBARS was detected only in the CE group. The content of TBARS in the red portion of *gastrocnemius* was significantly higher in the CE group than in the AE group. Moreover, in all the remaining tissues the

content of the TBARS after the exercise tended to be lower in the AE group as compared to the CE group.

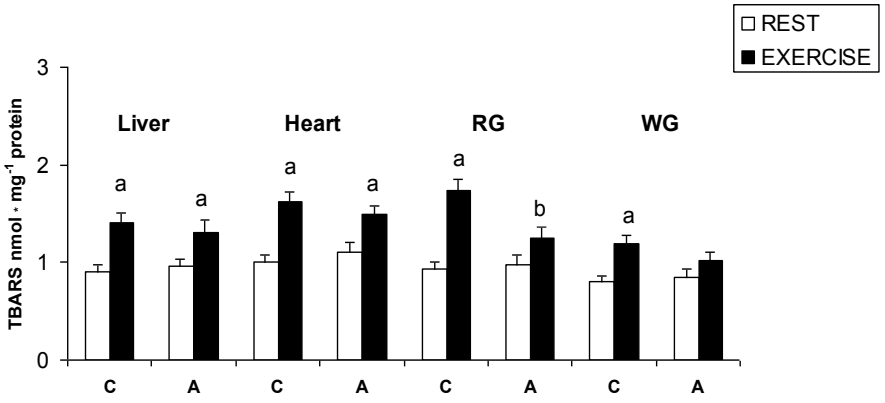

Fig. 1
Content of TBARS in the liver, heart muscle, and the red and white portion of the *gastrocnemius* muscle of the control rats (C) and rats given the extract from fruits of *Aronia melanocarpa* (A) at rest and after the exercise
a – significantly different from the resting value ($P<0.05$); b – significantly different from the control value ($P<0.05$).

The content of GSH in the examined tissues is shown in Fig.2. The analysis of variance revealed the significant effect of the AM administration on this content in the tested muscles (H, RG, WG) both at rest ($P<0.05$) and after the exercise ($P<0.01$). Despite that, no significant differences between the AR and CR groups were found at rest in the mean contents of GSH in the particular tissues, although the values of this index tended to be lower after the administration of AM. The exercise resulted in the significant decrease of the GSH concentration in each tissue obtained from the CE group. In contrast, no significant effect of the exercise on the GSH concentration in the tissues of animals from the AE group could be detected. After the exercise mean values of GSH in the heart and red portion of *gastrocnemius* were significantly higher in the AM-treated than in the control (C) rats, whereas in the white portion of *gastrocnemius* and in the liver these values tended to be higher in the AE as compared to the CE animals.

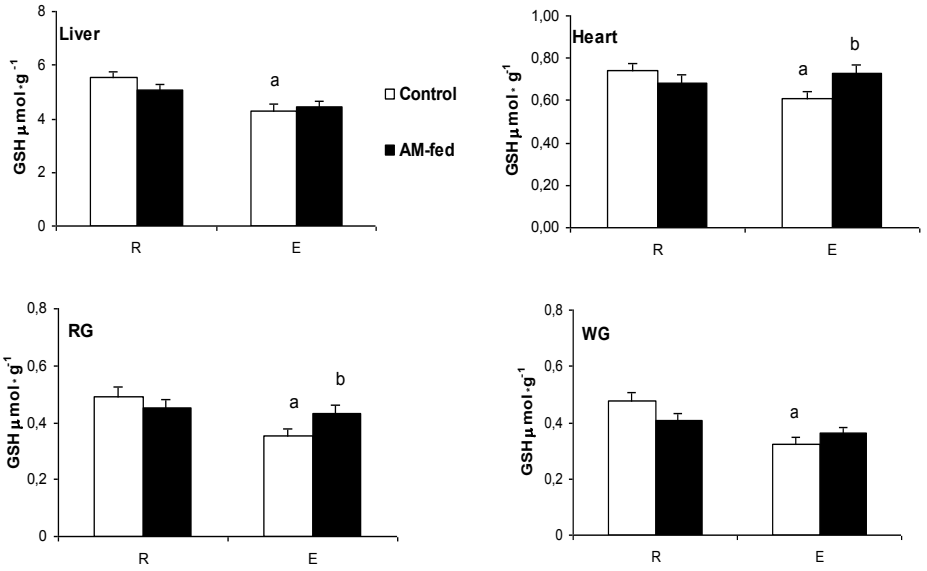

Fig. 2
Content of the reduced glutathione (GSH) in the liver, heart muscle, and the red (RG) and white (WG) portion of the *gastrocnemius* muscle of the control rats and rats given the extract from fruits of *Aronia melanocarpa* (AM-fed) at rest (R) and after the exercise(E)
a – significantly different from the resting value ($P<0.05$); b – significantly different from the control value ($P<0.05$)

Discussion

The described changes in the content of the reduced glutathione in the investigated tissues as a result of administration of the *Aronia melanocarpa* extract constitute an original finding of the present study. The directions of the changes seems to be different at rest and after the exercise. Following the administration of AM the GSH content at rest tended to decrease. In turn, the AM administration inhibited the exercise-induced decrease of GSH in each tissue tested, especially in the heart and RG.

Post-exercise decrease in the GSH level is one of the indices of the activity of reactive oxygen species. Dufaux *et al.* [3] in their experiments conducted on the moderately trained young men detected a decrease in the blood GSH concentration after the 2.5-hour run despite the lack of significant changes in the concentration of TBARS. The exercise-induced reduction of the GSH level has been frequently reported in the liver and muscles [22,27,29] as well as in the blood [3,4,10,21] of the tested subjects. Notably, however, in some studies exercising led to elevation of GSH in tissues [17,18]. The differences in the blood GSH content after the exercise can be related to variations in the level of training, intensity and time of the exercise as well as duration of the interval between completion of the exercise and the time of blood sampling.

Glutathione is a very important element of the antioxidative system of the body [16,19]. In the reaction catalysed by glutathione peroxidase glutathione reduces free oxygen radicals. Moreover, the enzyme scavenges hydroxyl radicals and singlet oxygen. It also reduces tocopherol radicals preventing thereby lipid peroxidation. The oxidised form of GSH can in turn be reduced by glutathione reductase in cooperation with NADPH. Stores of GSH are replenished through the *de novo* synthesis in the liver. GSH is released from the liver to the serum wherefrom it is picked up by working muscles. Presumably, during the prolonged exercise to which the rats were exposed in our study, the mechanisms of the GSH homeostasis appeared inefficient. As a result, the level of the reduced glutathione decreased. The inhibition of the AM-induced post-exercise reduction of GSH points to a marked antioxidative activity of the tested preparation.

A slightly weaker antioxidative activity of AM was detected when TBARS was used as the index of lipid peroxidation. Still, this activity was somewhat stronger than that reported in our previous investigation [9] in which AM was administered at half the dose used in the present study. Thus, inhibition of the AM-induced post-exercise elevation of TBARS was found not only in the red portion of the *gastrocnemius* muscle, as reported previously by us, but also in the white portion of the muscle. However, significant differences between the CE and AE groups in the post-exercise content of TBARS were detected only in RG even though this content tended to be lower also in other tissues obtained from the AE compared to the CE animals.

The demonstrated in the present study antioxidative activity of the AM extract should encourage consumption of the *Aronia* fruits or the *Aronia*-derived products as the effective antioxidants. Importantly, as indicated by the results of the published studies, these substances seem to be devoid of any deleterious side effects. Indeed, the above cited epidemiological data clearly suggest that

consumption of anthocyanins and other flavonoids prevents the development of numerous diseases [8,13,15,20]. Of importance is also the fact that the active substances tested by us are natural components of many food products, predominantly fruits and vegetables.

It is noteworthy that the herewith described effect of AM on the GSH content at rest differed from that detected after the exercise. The analysis of variance revealed the significant effect of the AM administration on the GSH content in the tested muscles (H, RG, WG) at rest. Although no significant differences between the AR and CR groups were found in the mean contents of GSH, the values of this index in all the tissues tested tended to be lower after the administration of AM. It is likely that in the environment of the low concentration of reactive oxygen species anthocyanins exhibit a mild pro-oxidant activity. In fact, some flavonoids and other antioxidants were shown to act that way in the *in vitro* systems [11,12,26]. It is also possible that during rest, when relatively small amounts of reactive oxygen species are being generated, administration of antioxidants, capable of interacting in the tissues with glutathione, α-tocopherol, and other antioxidants [1,7,14], reduces the muscle requirement for GSH leading thereby to its enhanced release from the muscles or its lowered uptake from the serum. This suggestion is supported by our present finding, that the tendency of the GSH content to decrease in the tested tissues was not accompanied by a tendency of TBARS to elevate in these tissues.

In conclusion, the obtained results show that prophylactic application of the *Aronia melanocarpa* extract mitigates the exercise-induced reduction of the GSH concentration as well as the elevation of the TBARS content in the tissues of the investigated animals.

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