Institute of Experimental Morphology, Pathology and Anthropology with Museum
Bulgarian Anatomical Society

Acta morphologica et anthropologica, 22
Sofia ● 2015

Morphology

Vasoprotective Properties of Aronia Melanocarpa – a Histological and Morphometric Study

E. Daskalova[1], S. Delchev[1], I. Bivolarski[2], P. Denev[3,4], M. Kratchanova[3,4], P. Cvetkov[5], M. Kaluch[5]

[1] *Department of Anatomy, Histology and Embryology, Medical University of Plovdiv, Bulgaria*
[2] *Department of General and Clinical Pathology, Medical University of Plovdiv, Bulgaria*
[3] *Laboratory of Biologically Active Substances, Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Plovdiv, Bulgaria*
[4] *ITC – Innovative-Technological Centre Ltd., Plovdiv, Bulgaria*
[5] *Medical University of Plovdiv, Plovdiv, Bulgaria*

The social significance of age-related diseases is determined by their global role in mortality and morbidity, particularly in economically developed countries. Changes in elastic and muscular arteries walls, resulting from age-related restructuring and progression of atherosclerotic lesions, underlie coronary heart disease and cerebrovascular disease. Their prevention through administration of natural products is a research area with huge potential, and application of natural antioxidants is one of the leading strategies to retard vascular aging. *Aronia melanocarpa* juice is a rich source of polyphenols and is characterized by very high antioxidant activity *in vitro*. The aim of the current study was to investigate the effect of aronia juice intake on age-related vascular changes of rat aortic walls. We used a model of aging male rats, whose thoracic aorta walls were subjected to macroscopic, histological (hematoxilyn-eosin), histochemical (orcein) and morphometric studies. The comparative analysis between the target group of old animals supplemented with aronia juice; young untreated rats and old controls (not supplemented), revealed that aronia-supplemented animals were characterized with reduced atherosclerotic lesions and a lower level of restructuring of aortic walls. These data confirm that *Aronia melanocarpa* juice successfully retards age-related vascular aging, and can be recommended as a prophylactic tool for healthy aging.

Key words: vascular aging, antioxidants, Aronia melanocarpa.

Introduction

Age-related diseases are social problem with global significance and their prevention through natural products is a research field with great potential. Cardiovascular damage is among the main causes of morbidity and mortality in relation to the aging process.

The morphological changes of the wall of the large arteries, including the aorta are result from age-related restructuring and virtually create pathophysiological conditions for deterioration of their functions leading to pathological changes [9]. The application of antioxidants is one of the strategies to slow the process of vascular aging [4, 5, 6, 7, 8]. *Aronia melanocarpa* juice is a rich source of polyphenols and as such exhibits a very high antioxidant activity [2].

The aim of the current experimental study was to investigate the effect of *Aronia melanocarpa* intake in the age-related vascular changes in the aortic wall.

Materials and Methods

Black chokeberry (Aronia melanocarpa) fruit juice: Commercially available sterilized black chokeberry juice, packed in glass bottles (250 ml), was provided by Vitanea Ltd, Plovdiv, Bulgaria.

In the experiment 22 male Wistar rats were divided into three groups. 14 of them aged 10 months with initial body weight 418 ± 57 g were divided into 2 groups: **control old (CO)**, which were on a standard diet and tap water *ad libitum*, and **Aronia group (A)**, which received *ad libitum* chokeberry juice diluted 1:1 in drinking water and a standard rodent chow. The daily dose of fruit juice ingested by the animals was 10 ml/kg. The **control young group (CY)** consisted of 8 animals aged 2 months with body weight 147 ± 12 g. The experiment lasted 90 days. The experimental protocol was approved by the Committee on Ethical Treatment of Animals of the Bulgarian Agency for Food Safety. In the end of experiment animals were euthanized with i.m. Ketamin/Xilazine and the thoracic aortas were separated and prepared for examination.

Macroscopic and histology examination: Descending thoracic aortas from CY, CO and A groups of rats were harvested and fixed in 10% formalin. Aorta thoracica was cleared of the visible connective tissue, cut longitudinally and pressed between two glass slides. Parts of fixed aortic segments were embedded in paraffin and sectioned at 5 μm. Sequential sections were stained with hematoxylin/eosin and orcein. The photomicrographs enclosed were taken on Nikon Microphot SA microscope (Japan), equipped with a Camedia-5050Z digital camera (Olympus, Japan).

Morphometric analysis: All quantitative image analysis was performed using software"DP-Soft" 3.2, Olympus, Japan. For each study, analysis of histology sections was performed using at least 5 randomly chosen high-power fields from 5 different sections from each young (n = 8) and old (n = 7) rat. The thickness of aortic tunica media (μm) was measured from the internal elastic lamina to the adventitial border. Cell density (cells/50μm^2) in the tunica media of aortic wall was calculated as mean by counting the number of nuclei in 50/50μm areas of tunica media tissue from 5 sections from every young (n = 8), old (n = 7) and Aronia supplemented (n = 7) rat.

Statistical analysis: The results were analyzed with SPSS 13.0 statistical program. Statistical significance between experimental groups was determined by Student's t-test and differences were considered significant at $P < 0.05$. The intergroup comparison was made with one-way ANOVA.

Results

From gross appearance of the intimal surface of the longitudinal section of thoracic aorta (**Fig. 1A**) was evident that there were no visible pathological changes in the intima of the thoracic aorta in the group of young controls. In the group of elderly controls

(**Fig. 1B**), initial fibrous plaques and focal hemorrhages in atherosclerotic plaques were observed. There were also single lipid stripes and spots. In aronia-supplemented group

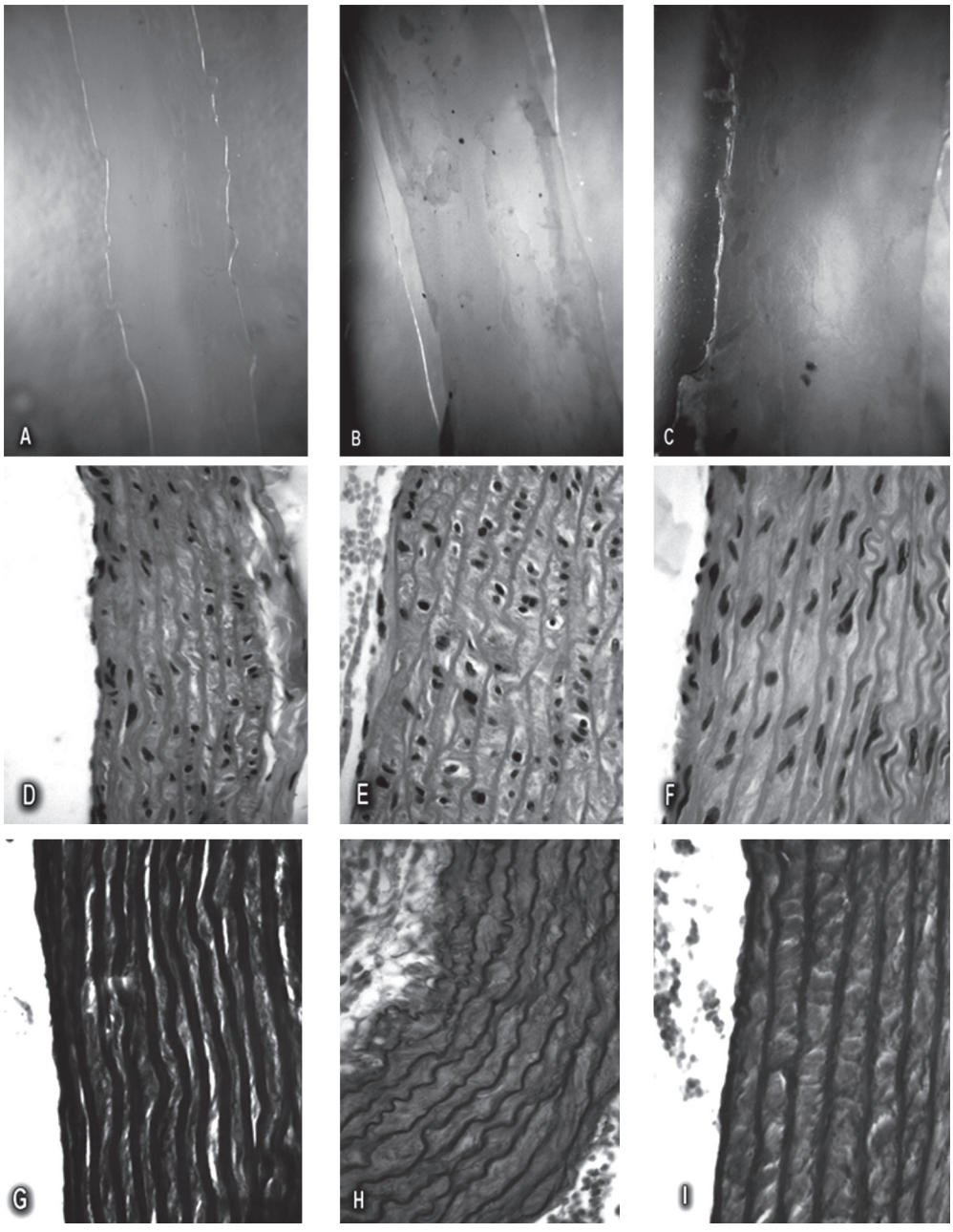

Fig. 1. Gross appearance of the intimal surface of the longitudinal section of thoracic aorta of the experimental groups: **A:** controls young, **B:** controls old, **C:** Aronia supplemented group, (magn. × 20), **Hematoxylin/eosin staining D:** controls young, **E:** controls old, **F:** Aronia supplemented group, (magn. × 400), **Orcein staining G:** controls young , **H:** controls old, **I:** Aronia supplemented group, (magn. × 400)

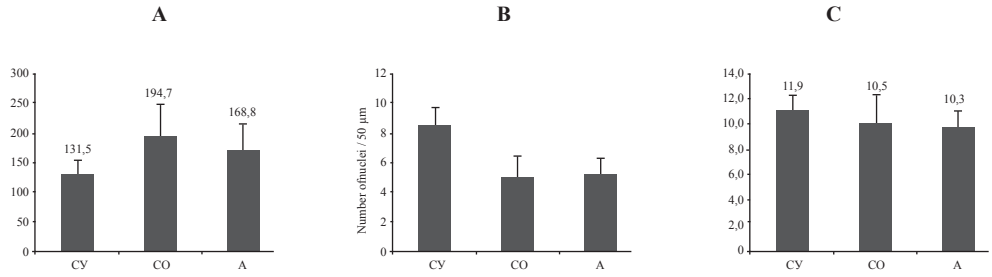

Fig. 2. A: Thickness of aortic tunica media, CY in comparison with CO $p < 0.05$; **B:** Cell density of aortic tunica media, CY in comparison with CO $p < 0.05$; **C:** Number of elastic membranes in aortic tunica media

(**Fig. 1C**) the intima was smooth and homogeneous and only single lipid stripes and spots were observed.

The hematoxylin/eosin stainings of thoracic aorta of the experimental groups are shown in **Fig. 1D, E, F**. In the cross-sections of aorta of the young controls (**Fig. 1D**), the tunica intima was smooth without abnormal depositions, the tunica media was presented with evenly and parallel elastic membranes. Their thickness and density were preserved. Smooth muscle cells, located between the elastic membranes were normal in size, shape and density, with normochromatic spindle cores. Tunica adventitia was represented by the usual loose connective tissue. In the crosscut of aorta of the old controls (**Fig. 1E**) the tunica intima was with uneven edges with focal plaque-like thickening. The tunica media was expanded, elastic membranes were separated, and considerably loosen and thinned, and bended serpentine. Smooth muscle cells were enlarged, with perinuclear vacuoles and pale cytoplasm. The nuclei of the cells were reduced in size, with polymorphism and polihromaziya. Some of them had pyknotic form, their orientation was transverse relative to the elastic membrane. In the crosscut of aorta of aronia group (**Fig. 1F**), the tunica intima was smooth, with protrusion of single endothelial cells to the lumen. The tunica media was presented by evenly placed parallel elastic membranes in the inner half of the aorta and serpentine bended – in the outer half. The thickness of the elastic membranes was slightly reduced and their density was stored. Smooth muscle cells had a normal spindle shape, longitudinally oriented, with preserved size, shape and density.

From **Fig. 1G** it is evident that tunica media in young controls was represented by smooth, thick elastic membranes, intensely dyed by orcein. The orcein staining in old controls (**Fig. 1H**) showed loosening and fragmentation of the paler-colored elastic membranes. Orcein staining in the Aronia group (**Fig. 1I**) showed smooth and straight elastic membranes with preserved integrity and staining intensity closer to that of young controls.

Data in **Fig. 2** demonstrated the results of the morphometric study of tunica media of aorta thoracica**.** Regarding the thickness of tunica media (**Fig. 2A**), statistically significant differences were established between younger and older controls ($p < 0.05$). Differences between old controls and aronia group did not reach statistical significance. In terms of the number of nuclei in tunica media (**Fig. 2B**), statistically significant differences were detected only between younger and older controls ($p < 0.05$). Regarding the number of the elastic membrane in the tunica media, differences between groups did not reach statistical significance.

Discussion

Our findings demonstrate convincingly that in aronia-supplemented animals, age-related changes are discrete and structure of the vascular wall is visibly preserved. This finding demonstrates the delay in the age changes and the preservation of the vessel wall under the influence of supplementation with Aronia melanocarpa juice.

Our data confirm that with advancing age the thickness of the aortic tunica media is increasing statistically significant [9]. Supplementation with Aronia juice leads to a reduction in its dimensions, though not significantly.

Our data confirms findings from other authors [1, 9] that with increased age, a decreased quantity of smooth muscle cells is found inside the tunica media, which are responsible for synthesizing elastin within the aorta.

As recognized by other authors, the amount of elastic membrane does not change with age [1, 3, 9]. Our results confirm the findings that the quantity of the elastin membranes remains inchanged during aging on account of the deterioration of their quality, which is clearly demonstrated by staining with orcein. However, as it is evident from the microphotographs, their quality visibly deteriorates in untreated adult controls and partially improves in aronia-treated rats.

Age-related physiological changes to the aorta are associated with a progressive decline in the elastic properties of the aortic wall [1, 3]. Greenwald [3] reported that conduit arteries become stiffer with age because elastin becomes fragmented, degraded and replaced by much stiffer collagen. Furthermore, both proteins become stiffer because of cross-linking and calcification, and these changes are accelerated by uraemia, hyperglycaemia and oxidative stress [10]. Our results demonstrates that supplementation with aronia melanocarpa juice preserves the structure of the elastic membranes.

Conclusion

Age-related structural changes in the aortic wall lead to increased aortic stiffness, increasing its diameter and length, reducing its compliance and contractility of the aortic wall [1, 3]. These amendments violate its functions and are a prerequisite for the emergence of pathologies, some of them fatal. Preservation of aortic wall and delay of age-related changes are an essential preventive measure for cardiovascular diseases such as hypertension, aortic dissection, aneurysm and rupture, congestive heart failure, complications of ischemia and stenosis. Achieving this with non-pharmacological agents is one of the latest strategies in the fight against aging. As a functional food juice, *Aronia melanocarpa* shows convincing vaso-protective properties and can be recommended as a prophylactic tool for healthy aging.

References

1. **Collins, J. A., J. V. Munoz, T. R. Patel, M. Loukas, R. S. Tubbs.** The anatomy of the aging aorta. – Clin. Anat., **27**, 2014, 463-466.
2. **Denev, P., Ch. Kratchanov, M. Ciz, A. Lojek, M. Kratchanova.** Bioavailability and antioxidant activity of Black chokeberry (Aronia melanocarpa) polyphenols: in vitro and in vivo evidences and possible mechanisms of action: a review. – Compr. Rev. Food Sci. F., Vol. **11**, 2012, 472-489.
3. **Greenwald, S. E.** Ageing of the conduit arteries. – J. Pathol., **211**, 2007, 157-172.
4. **Medina-Remón, A., R. Estruch, R. M. Lamuela-Raventós, A. Vallverdú-Queralt**. The effect of polyphenol consumption on blood pressure. – Mini Rev. Med. Chem., **13**, 2013, 1137-1149.

5. **Miquel, J.** Nutrition and ageing. – Public Health Nutr., **4**, 2001, 1313-1314.
6. **Moline, J., I. F. Bukharovich, M. S. Wolff, R. Phillips.** Dietary flavonoids and hypertension: is there a link? – Med. Hypotheses, **55**(4), 2000, 306-309.
7. **Pase, M. P., N. A. Grima, J. Sarris.** The effects of dietary and nutrient interventions on arterial stiffness: a systematic review. – Am. J. Clin. Nutr., **93**, 2011, 446-454.
8. **Sanchez-Roman, I., G. Barja.** Regulation of longevity and oxidative stress by nutritional interventions: Role of methionine restriction. – Exp. Gerontol., **48**, 2013, 1030-1042.
9. **Wheeler, J. B., R. Mukherjee, R. E. Stroud, J. A. Jones, J. S. Ikonomidis.** Relation of murine thoracic aortic structural and cellular changes with aging to passive and active mechanical properties. – J. Am. Heart Assoc., **4**(3), 2015 (in press).
10. **Zarkovic K., P. Larroque-Cardoso, M. Pucelle, R. Salvayre, G. Waeg, A. Nègre-Salvayre, N. Zarkovic.** Elastin aging and lipid oxidation products in human aorta. – Redox. Biol., **4**, 2015, 109-117.