Research Article

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Effects of the *Aronia melanocarpa* extract action on the activity of mitochondrial creatine kinase under immobilization stress in old rats

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ABSTRACT

The effects of the *Aronia melanocarpa* extract on mitochondrial creatine kinase isoenzyme of the old rats heart under stress were studied. The research was performed on 30 male rats of the Wistar line. Old (22–25 months) animals were used for the experiment. It was established, that the injection of the *Aronia melanocarpa* extract at a dose of 0.2 g/kg 60 minutes before the immobilization has limited sensitivity of the heart muscle's CPK-MT to damaging stress factors (reduced medium pH, increased medium tonicity, increased concentration of calcium, activated free radical processes), and helps the normalization of its kinetic properties, has an influence on the myocardium's kinetic supply. Thus, the extract of *Aronia melanocarpa* increases the myocardial resistance to the injury effect of stress.

Keywords: stress, myocardium, aging, *Aronia melanocarpa* extract, mitochondrial creatine kinase.

1. Introduction

Cardiovascular diseases such as myocardial ischemia, arrhythmias, coronary, and cardiosclerosis may be caused by complex of etiological factors including stress [1–4]. Recent studies have revealed that aging is reducing the adaptive features of the body [5, 6, 7], resulting in reduction of its resistance to stress factors [5]. Therefore, the elaboration of medications with expressed cardioprotective and antistress activity is a current issue of medical and pharmaceutical science. Natural medications, including those that obtained from *Aronia melanocarpa* are among the most promising representatives of remedies with the above-mentioned pharmacological activity.

A. melanocarpa contains the wide spectrum of biologically active components that can be used as protective compounds against injurious effects of environment. Anthocyanidins, proanthocyanidins, flavonoids and ascorbic acid are among the most pharmacologically valued compounds present in abovementioned plant [8-18]. The high antioxidant activity, cell protective activity in conditions of induced apoptosis and mitochondrial disfunction [9] and antibacterial activity [10, 21, 22] of *Aronia* fruits extract according to previously reported results are associated namely with the presence of anthocyanidins [10-14, 19, 20, 23].

Extracts of *Aronia* reveal significant anti-inflammatory activity. It`s administration decreases the blood level of inflammation markers (IL-β, TNF-α) [12, 14, 24]. Biologically active components of *Aronia* extracts inhibited RANKL – induced osteoclast differentiation [25].

The cyanidins that present in *Aronia* fruits reveal positive effect on numerous metabolic dysfunctions including dyslipidemia and hyperglycemia [12, 26]. These compounds retard the lipids accumulation *via* stimulation of lipolysis and inhibition of phosphodiesterase [27]. Abovementioned facts allow to recommend the *Aronia* extracts at treatment of diabetes, hypertonia, and cardiovascular system diseases [12, 26, 28].

It should be noted that available information about their cardiotropic effect development mechanism is not enough for it`s total understanding. One of the theories associates the pharmacological activity of *A. melanocarpa* preparations with their effects on the state of the lipid bilayer of cardiomyocyte membranes, as well as on their function and structure [29].

Visceral organs tissue is unequally sensitive to stress-associated damaging factors, therefore, in old age the cardiovascular system diseases, which are known as age-related pathology dominate [17, 30, 31].

Violation of myocardial energy supply plays an important role in the formation of stress myocardial damage [6, 32-33]. The definition of the mitochondrial creatine phosphokinase (CPK-MT) functioning - an enzyme that ensures the recovery of cardiomyocytes energy reserves [7, 34], have a great importance in this case. Therefore, we can suggest that the correction of the enzyme function would also diminish the stress - associated heart damage.

Thus, present work is aimed to study of the *A. melanocarpa* extract effect on the activity of mitochondrial creatine kinase cardiomyocyte under immobilization stress in old rats.

The aim of the work is to investigate the effects of a perspective phytopreparation on the CPK-MT properties of old rats under stress.

2. Material and Methods

2.1. Preparation of the extract

The *A. melanocarpa* leaves were collected from cultivated plants in Zaporizhzhia region of Ukraine. 100 g of lyophilized leaves were treated by 500 ml of 80% aqueous solution of ethyl alcohol at 78°C under stirring for 30 minutes. The plant material was filtered off and ethyl alcohol was evaporated from the filtrate under vacuum. The chlorophyll and lipophilic compounds were removed *via* extraction by petroleum ether. Then biologically active compounds were extracted from aqueous solution by ethyl acetate-ethanol mixture (8:2). Organic solvents were evaporated from the obtained mixture to give dry extract that was studied [20, 29].

2.2. Pharmacological studies

Thirty Wistar male rats were used in the study. Animals were kept in constant environmental conditions (20 °C,12-h light/dark cycle) and were on a standard laboratory diet.

Animals were divided into groups: 1 – intact rats, 2 – control rats that were affected by immobilization

stress by fixing them in a dorsal position for 30 minutes, 3 – rats that were intraperitoneally administered with the *A. melanocarpa* extract, 0.2 g/kg, 60 minutes before the immobilization [20]. The emergence of stress was verified pathomorphologically and by evaluation of the level of glucocorticoid hormones (11-hydroxycorticosteroids) in the blood using the fluorimetric method by a spectrofluorimeter Hitachi MPF-4 (Japan) [35].

The study was conducted in accordance with the requirements of the European Council Directive of November 24, 1986, for Care and Use of Laboratory Animals (86/609/EEC) [36], and according to the general ethical principles of experiments on animals adopted by the First National Congress of Ukraine on Bioethics (2001), as well as other international agreements and legislation of Ukraine in this area (Protocol No.2, approved 21.12.2022 by Bioethics commission of Zaporizhzhia National University).

After completing of immobilization, animals were decapitated using guillotine under anesthesia by ether. The heart was extracted and washed from the blood. The left ventricular myocardium was isolated and homogenized with 0.25M sucrose and 0.01M Tris (pH 7.4) in a glass Potter-Elvehjem homogenizer. Homogenate of cardiac muscle was filtered through 4 layers of gauze and centrifuged at 1000g for 10 minutes. The supernatant was centrifuged again at 10000g for 20 minutes. The washed precipitate was a crude mitochondrial fraction. All procedures were performed at 4-6^{0}C. Enzymological method of a CPK activity [5] was used in the studies. Lowry method was used to determine total protein content [37]. Statistical analysis of the results was performed using a nonparametric method of Wilcoxon – Mann – Whitney.

3. Results and Discussion

According to a study, significant changes in the activity of myocardial CPK-MT under stress was not observed. Mitochondrial isoenzyme CPK activity was increased, as we considered as result of the changes in balance of free and membrane - conjugated forms of isoenzyme in the mitochondrial fraction under stress [7]. Considered the fact that stress – associated tissue hypoxia, results the changes of the inner structure of the tissue heart [38], it was interesting to explore some kinetic properties of MT-CPK. Experimental data showed, that the sensitivity of the studied enzyme to decreasing of acidosis (Table 1), increasing of its tonicity (Table 2), as well as the inhibiting effect of calcium ions (Table 3) increases under stress.

It is known, that mentioned above factors are particularly important in the formation of the tissue metabolic response to the action of stress agents. Therefore, we can suggest that the conditions for limiting the activity of this enzyme are formed in cardiomyocytes under stress in vivo. It creates significant barriers for transporting macroergic compounds in the myocardial cells and leads to the violation of muscle contraction energy supply under stress, as well as to the development of stress myocardial damage.

Animals were injected with *A. melanocarpa* extract for 60 minutes to model immobilization stress for correcting in the properties of CPK-MT. Prevention of the emergence of increased activity of the enzyme in the mitochondrial fraction is one of the arguments which proved the effects *A. melanocarpa* extract on the interaction of the enzyme with the surface of inner membrane of mitochondria. Moreover, the preparation showed a reducing effect in relation to the inhibitory effect of a pH decrease (Table 1), environmental tonicity (Table 2), and inhibitory effect of calcium ions (Table 3).

Conducted study proved the ability of studied *A. melanocarpa* extract to reduce the stress-associated effects on the CPK-MT. Under the condition of stress arising during immobilization of animals, prerequisites are formed in the heart muscle for stimulating the production of reactive oxygen in mitochondria. As a result, they increase the rate of free radical processes associated with the oxidation of proteins, lipids, nucleic acids, etc. [33, 39, 40]. At the same time, in the polypeptide chains of proteins, including mitochondrial creatine kinase, the side chains of amino acids are oxidized. That leads to a change in the conformation of its molecule. This is accompanied by modulation of its catalytic and regulatory properties, which predetermine the disruption of energy transport from mitochondria to myofibrils of cardiomyocytes. The energy supply of the myocardium decreases and the strength of heart contractions decreases, as a result of the formation of all these shifts in immobilized animals. That is a characteristic manifestation of stress heart dysfunction [6, 32-33].

The introduction of *Aronia* extract to animals limits the dysfunction of the heart during immobilization

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Table 1. Effects of the *Aronia melanocarpa* extract on the activity of CPK-MT of old rats heart in different environmental pH value (M±m)

pH	CPK-MT activity, ATP mmol/ mg protein/ min.		
	Intact (n=10)	Stress (n=10)	Extract +stress (n=10)
7.4	0.30±0.01	0.38±0.04	0.25±0.02
7.0	0.24±0.01	0.19±0.01[a]	0.16±0.04
6.5	0.21±0.01	0.13±0.03[a]	0.9±0.02[a]

[a] significant deviation relative to intact animals ($p < 0.05$)

Table 2. Effects of the *Aronia melanocarpa* extract on the dependence of the old rats heart CPK-MT activity of the environmental tonicity under stress (M±m)

NaCl concentration (mmol)	CPK-MT activity, ATP mmol/ mg protein/ min.		
	Intact (n=10)	Stress (n=10)	Extract +stress (n=10)
0	0.30±0.01	0.38±0.04	0.25±0.02
120	0,42±0.03	0.22±0.01[a]	0.27±0.06[a]

[a] significant deviation relative to intact animals ($p < 0.05$)

Table 3. Effects of the *Aronia melanocarpa* extract on the old rats heart CPK-MT activity in presence of the $CaCl_2$ in environment (M±m)

$CaCl_2$ concentration (mmol)	CPK-MT activity, ATP mmol/ mg protein/ min.		
	Intact (n=10)	Stress (n=10)	Extract +stress (n=10)
0	0.30±0.01	0.38±0.04	0.25±0.02
120	0.25±0.02	0.18±0.02[a]	0.16±0.02[a]

[a] significant deviation relative to intact animals ($p < 0.05$)

stress. This is due to the fact that it contains numerous antioxidants that act as free radical scavengers. These include the previously mentioned anthocyanidins, proanthocyanidins, flavonoids, as well as ascorbic acid [8–18]. Moreover, it was shown [41] that the preliminary administration of *Aronia* extract significantly limits the accumulation of TBARS and is the reason for the decrease in the level of reduced glutathione in rats subjected to intense exercise. By increasing the antioxidant activity of the cardiac muscle, these substances limit the formation of oxidative stress in it during animal immobilization. Due to this, there is a decrease in free radical oxidation of the CPK-MT molecule. And hence the prevention of mitochondrial dysfunction and disruption of the energy supply of the heart muscle is present. All this reflects the prospects of using *Aronia* extract as a drug that limits stress damage to the myocardium. In all likelihood, this drug has a similar effect on other tissues of the internal organs, and therefore it can be assumed that it has an anti-stress effect in general.

At the same time, the question remains as to which components of *Aronia* extract provide its protective effect on the myocardium under stress? It is likely, that these include not only antioxidants, but also substances that have a membrane-stabilizing effect, as well as stimulating the synthesis of ATP in mitochondria. Our further studies will be devoted to the study of this issue.

4. Conclusions

According to studies of mitochondrial creatine kinase activity it was found the sensitivity of the enzyme to decreased of pH of medium, increased tonicity and to inhibitory influence of calcium ions increase under stress.

After injection of *A. melanocarpa* extract, in the formation of stress, the increase of the mitochondrial CPK activity was not determined. This may be due to the ability of preparation to limit the oxidative modification of enzyme under stress.

Extract of *A. melanocarpa* improved the energy supplement at a dose of 0.2 g/kg 60 minutes before the immobilization. *A. melanocarpa* extract may be used for the normalization sensitivity of CPK-MT to stress associated damage factors in the heart muscle.

According to the studies, the experiments with *A. melanocarpa* extract need to be continued because they are significant and perspective as they allow to determine the influence of damaging factors in aging.

Conflict of Interest

The authors declare no conflict of interest.

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Statement of Contribution of Researchers

Concept – V.S., V.D. Design – V.S., Supervision – V.S., Resources H.M., H.B., I.N., O.B., Materials –H.B., I.N., O.B., Data Collection and/or Processing – H.M., H.B., I.N. O.V., Analysis and/or Interpretation – V.S., H.M., H.B., I.N. O.V., Literature Search – V.S., O.V., O.B., Writing – V.S., O.V. Critical Reviews – V.S., H.M., V.D.

References

1. Song H, Fang F, Arnberg FK, Mataix-Cols D, Fernández de la Cruz L, Almqvist C, et al. Stress related disorders and risk of cardiovascular disease: population based, sibling controlled cohort study. BMJ. 2019;365:l1255. https://doi.org/10.1136/bmj.l1255
2. Steptoe A, Kivimäki M. Stress and cardiovascular disease. Nat Rev Cardiol. 2012;9(6):360-70. https://doi.org/10.1038/nrcardio.2012.45
3. Fujino Y, Tanabe N, Honjo K, Suzuki S, Shirai K, Iso H, et al. A prospective cohort study of neighborhood stress and ischemic heart disease in Japan: a multilevel analysis using the JACC study data. BMC Public Health. 2011;11:398. https://doi.org/10.1186/1471-2458-11-398
4. Davydov VV, Shvets VN. Adenine nucleotide and creatine phosphate pool in adult and old rat heart during immobilization stress. Gerontology. 2002;48(2):81-3. https://doi.org/10.1159/000048931
5. Fedosov SN, Belousova LV. Vliianie oligomerizatsii na svoĭstva sushchestvennykh dlia aktivnosti SH-grupp v mitokhondrial'noĭ kreatinkinaze [Effect of oligomerization on the properties of essential SH-groups of mitochondrial creatine kinase]. Biokhimiia. 1988;53(4):550-64
6. Nagornaya NV, Chetverik NA, Fedorova AA. Energetic exchange in cell in norm and patology. Possibility of it`s estimation. Clinical Herontology. 2008;6:58.
7. Davydov VV, Shvets VN. Differential changes in the properties of mitochondrial isoenzyme creatine kinase from heart of adult and old rats during stress. Exp Gerontol. 1999;34(3):375-8. https://doi.org/10.1016/s0531-5565(99)00020-0
8. Teneva D, Pencheva D, Petrova A, Ognyanov M, Georgiev Y, Denev P. Addition of medicinal plants increases antioxidant activity, color, and anthocyanin stability of black chokeberry (*Aronia melanocarpa*) functional beverages. Plants (Basel). 2022;11(3):243. https://doi.org/10.3390/plants11030243
9. Meng L, Xin G, Li B, Li D, Sun X, Yan T, et al. Anthocyanins extracted from *Aronia melanocarpa* protect SH-SY5Y cells against Amyloid-beta (1-42)-induced apoptosis by regulating Ca^{2+} homeostasis and inhibiting mitochondrial dysfunction. J Agric Food Chem. 2018;66(49):12967-77. https://doi.org/10.1021/acs.jafc.8b05404
10. Denev P, Číž M, Kratchanova M, Blazheva D. Black chokeberry (*Aronia melanocarpa*) polyphenols reveal different antioxidant, antimicrobial and neutrophil-modulating activities. Food Chem. 2019;284:108-17. https://doi.org/10.1016/j.foodchem.2019.01.108
11. Sidor A, Gramza-Michałowska A. Black chokeberry *Aronia melanocarpa* L.-A qualitative composition, phenolic profile and antioxidant potential. Molecules. 2019;24(20):3710. https://doi.org/10.3390/molecules24203710
12. Jurikova T, Mlcek J, Skrovankova S, Sumczynski D, Sochor J, Hlavacova I, et al. Fruits of black chokeberry *Aronia melanocarpa* in the prevention of chronic diseases. Molecules. 2017;22(6):944. https://doi.org/10.3390/molecules22060944
13. Yang H, Kim YJ, Shin Y. Influence of ripening stage and cultivar on physicochemical properties and antioxidant compositions of Aronia grown in South Korea. Foods. 2019;8(12):598. https://doi.org/10.3390/foods8120598

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14. Bushmeleva K, Vyshtakalyuk A, Terenzhev D, Belov T, Parfenov A, Sharonova N, et al. Radical scavenging actions and immunomodulatory activity of *Aronia melanocarpa* propylene glycol extracts. Plants (Basel). 2021 Nov 15;10(11):2458. https://doi.org/10.3390/plants10112458

15. Krga I, Milenkovic D. Anthocyanins: From Sources and Bioavailability to Cardiovascular-Health Benefits and Molecular Mechanisms of Action. J Agric Food Chem. 2019;67(7):1771-83. https://doi.org/10.1021/acs.jafc.8b06737

16. Cvetanović A, Zengin G, Zeković Z, Švarc-Gajić J, Ražić S, Damjanović A, et al. Comparative *in vitro* studies of the biological potential and chemical composition of stems, leaves and berries *Aronia melanocarpa*'s extracts obtained by subcritical water extraction. Food Chem Toxicol. 2018;121:458-66. https://doi.org/10.1016/j.fct.2018.09.045

17. Staszowska-Karkut M, Materska M. Phenolic composition, mineral content, and beneficial bioactivities of leaf extracts from black currant (*Ribes nigrum* L.), raspberry (*Rubus idaeus*), and aronia (*Aronia melanocarpa*). Nutrients. 2020;12(2):463. https://doi.org/10.3390/nu12020463

18. Vendrame S, Klimis-Zacas D. Potential factors influencing the effects of anthocyanins on blood pressure regulation in humans: A Review. Nutrients. 2019;11(6):1431. https://doi.org/10.3390/nu11061431

19. Rudic J, Jakovljevic V, Jovic N, Nikolic M, Sretenovic J, Mitrovic S, et al. Antioxidative effects of standardized *Aronia melanocarpa* extract on reproductive and metabolic disturbances in a rat model of polycystic ovary syndrome. Antioxidants (Basel). 2022;11(6):1099. https://doi.org/10.3390/antiox11061099

20. Cuvorova IN, Davydov VV, Prozorovskiĭ VN, Shvets VN. [Peculiarity of the antioxidant action of the extract from *Aronia melanocarpa* leaves antioxidant on the brain]. Biomed Khim. 2005;51(1):66-71.

21. Kim SS, Shin Y. Antibacterial and *in vitro* antidementia effects of aronia (*Aronia melanocarpa*) leaf extracts. Food Sci Biotechnol. 2020;29(9):1295-1300. https://doi.org/10.1007/s10068-020-00774-y

22. Deng H, Xue B, Wang M, Tong Y, Tan C, Wan M, et al. TMT-based quantitative proteomics analyses reveal the antibacterial mechanisms of anthocyanins from *Aronia melanocarpa* against *Escherichia coli* O157:H7. J Agric Food Chem. 2022;70(26):8032-8042. https://doi.org/10.1021/acs.jafc.2c02742

23. Wei J, Yu W, Hao R, Fan J, Gao J. Anthocyanins from *Aronia melanocarpa* induce apoptosis in Caco-2 cells through Wnt/β-Catenin signaling pathway. Chem Biodivers. 2020;17(11):e2000654. https://doi.org/10.1002/cbdv.202000654

24. Banach M, Wiloch M, Zawada K, Cyplik W, Kujawski W. Evaluation of antioxidant and anti-inflammatory activity of anthocyanin-rich water-soluble aronia dry extracts. Molecules. 2020;25(18):4055. https://doi.org/10.3390/molecules25184055

25. Ghosh M, Kim IS, Lee YM, Hong SM, Lee TH, Lim JH, Debnath T, Lim BO. The effects of *Aronia melanocarpa* 'Viking' extracts in attenuating RANKL-induced osteoclastic differentiation by inhibiting ROS generation and c-FOS/NFATc1 signaling. Molecules. 2018;23(3):615. https://doi.org/10.3390/molecules23030615

26. Olechno E, Puścion-Jakubik A, Zujko ME. Chokeberry (*A. melanocarpa* (Michx.) Elliott)-A natural product for metabolic disorders? Nutrients. 2022;14(13):2688. https://doi.org/10.3390/nu14132688

27. Niesen S, Göttel C, Becker H, Bakuradze T, Winterhalter P, Richling E. Fractionation of extracts from black chokeberry, cranberry, and pomegranate to identify compounds that influence lipid metabolism. Foods. 2022;11(4):570. https://doi.org/10.3390/foods11040570

28. Middleton E Jr, Kandaswami C, Theoharides TC. The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. Pharmacol Rev. 2000;52(4):673-751.

29. Shvets V, Maslak H, Davydov V, Berest H, Nosulenko I. The effect of *Aronia melanocarpa* extract on the phospholipid composition of the rat myocardium during stress. Ceska Slov Farm. 2022;71(3):98-102.

30. Romano AD, Serviddio G, de Matthaeis A, Bellanti F, Vendemiale G. Oxidative stress and aging. J Nephrol. 2010;Suppl 15:S29-36.

31. Saner H. Stress als kardiovaskulärer Risikofaktor [Stress as a cardiovascular risk factor]. Ther Umsch. 2005;62(9):597-602. German. https://doi.org/10.1024/0040-5930.62.9.597.

32. Gavrysh OS, Shults NV, Kindzerska OL, Dorofeeva SI. Energetic supply of miocard at chronic heart failure of noncoronary genesis. Ukraine Cardiological Journal. 2011;3:44-9.

33. Meerson FZ. Pathogenesis and prevention of stress and ischemic heart lesion. Medicine. 1984;270.

34. Saks V, Dzeja P, Schlattner U. Cardiac system bioenergetics: metabolic basis of the Frank-Starling law. J Physiol. 2006;571(Pt 2):253-73.

35. De Moor P, Steeno O, Raskin M, Hendrikx A. Fluorimetric determination of free plasma 11-hydroxycorticosteroids in man. Acta Endocrinol. 1960;33:297–307.

36. European convention for the protection of vertebrate animals used for experimental and other scientific purposes. European Treaty Series 1986, No. 123. Strasbourg: Council of Europe.

37. Lowry O, Rosebrough N, Randall R, Farr A. Protein measurement with the folin phenol reagent. J Biol Chem. 1951;193:265–75.

38. Frolkis VV. The stress-age syndrome. Phisiologicheskii Juornal. 1991;37:3–11.

39. Shvets VN. Age-specific peculiarities of the accumulation of carbonylated proteins in subcellular fraction of myocardium under immobilization stress influence, Uchenye zapiski Tavricheskogo Natsionalnogo Universiteta im. V.I. Vernadskogo. Series "Biology, chemistry". 2008;21(1):169-73.

40. Cadenas E. Mitochondrial free radical generation, oxidative stress, and aging. Free Radic. Biol Med. 2000;29(3):222-30.

41. Faff J, Frankiewicz-Jozko A. Effect of anthocyanin pigments from fruits of *Aronia melanocarpa* on the exercise-induced increase in lipid peroxidation marker in rat tissues. Biology of Sport. 2003;20:15-23.

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