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RIBES NIGRUM FRUIT EXTRACT SHOWS CARDIOPROTECTIVE PROPERTIES IN EXPERIMENTAL ACUTE ISCHEMIC HEART DISEASE

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Abstract: The study was designed to assess antioxidant and cardioprotective properties of antioxidants-containing blackcurrant (*Ribes nigrum*) extract in experimental adrenaline-induced acute ischemic heart disease. The content of troponin-1 and myoglobin, the activity of creatine phosphokinase MB in blood serum and levels of TBA-reactive substances, reduced glutathione and ATP, as well as the activity of superoxide dismutase, catalase, alanine aminotransferase and aspartate aminotransferase in myocardial homogenates were determined in control intact animals and in rats with adrenaline-induced acute ischemic heart disease untreated and treated with the blackcurrant extract. Our findings suggest that the *Ribes nigrum* fruit extract administration at a dose of 0.1 ml / 100g of body weight results in activation of antioxidant system and energy metabolism in myocardium and normalization of redox metabolism.

Keywords: blackcurrant, antioxidants, anthocyanins, ischemic heart disease

INTRODUCTION:

Cardiovascular diseases are characterized by the highest prevalence and mortality. Despite the obvious trend towards a decrease in mortality rates among population of developed countries, cardiovascular diseases are still responsible for over 30% of all death cases in individuals older than thirty-five years (Sanchis-Gomar et al, 2016).

Numerous studies have focused on pathophysiology of ischemic heart disease (IHD). In particular, it has been reported that free radicals, including reactive oxygen species (ROS) and reactive nitrogen species (RNS), play a crucial role in pathogenesis of IHD (Csányi and Miller, 2014; Elahi et al, 2009). In addition to the ability of ROS to induce lipid peroxidation, DNA damage, protein oxidative modification, there is overwhelming evidence that ROS are involved in intracellular redox signaling (Schieber and Chandel, 2014). Overgeneration of ROS and RNS in IHD is observed against the background of the depressed antioxidant system leading to the development of oxidative stress (Cervantes Gracia et al, 2017; Vichova and Motovska, 2013). Such imbalance between pro- and antioxidants substantiates the feasibility of IHD antioxidant treatment. The possible ways of therapeutic interventions using antioxidants in patients with IHD have been studied for over three decades. The results of clinical trials focused on the use of antioxidant supplements for treatment of cardiovascular diseases seem to be disappointing (Leopold, 2015). However, given the role of oxidative stress in the pathophysiology of cardiovascular diseases, novel researches may identify novel antioxidants-based treatment strategies that will prove to be effective against cardiovascular pathology in the future. Thus, antioxidant-based therapeutic approaches aimed at ROS-mediated pathways still seem to be promising for the treatment of cardiovascular pathology.

The aim of the research was to evaluate antioxidant and cardioprotective effects of antioxidants-containing blackcurrant extract in experimental acute IHD.

MATERIALS AND METHODS:

Description of animals and groups

Thirty-five WAG male rats weighing 150-170 g kept in standard conditions of the vivarium were used in our experiment. They were randomly subdivided into 3 groups. The control group included 7 intact animals. Group 1 consisted of 14 rats with experimental acute adrenaline-induced IHD. They were subdivided into two subgroups: group 1.1 and group 1.2 with 7 rats in each. Rats from group 1.1 (n=7) were sacrificed on day 1 after the adrenaline injection, whereas rats from group 1.2 (n=7) were killed on day 3 after the adrenaline injection. *Ribes nigrum* fruit extract was orally administered to the animals from group 2 at a dose of 0.1 ml against the background of experimental acute IHD (n=14). They were also subdivided into two subgroups: group 2.1 and group 2.2 with 7 rats in each. Animals from group 2.1 (n=7) were sacrificed on day 1, while rats from group 2.2 (n=7) were killed on day 3 after disease induction. The extract was given two minutes after the adrenaline injection.

Acute ischemic heart disease modeling and sample collecting

Acute IHD was caused by an intraperitoneal injection of 0.1% adrenaline solution at a dose of 0.2 ml per 100 g of body weight (Denisov and Rukoveshnikova, 1999). Blood samples were taken from the caudal vein of animals 20 minutes after the adrenaline injection in order to assess the content of troponin-1 and myoglobin and the activity of creatine phosphokinase MB in blood serum. Rats were sacrificed on day 1 and day 3 after the adrenaline injection. Cranial blood samples were collected to obtain blood serum. Myocardium was collected to

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prepare homogenates and sections for morphological investigation.

Preparation of myocardial homogenates

Myocardial homogenates were prepared in accordance with the Severin's method (Meshkova and Severin, 1979). Myocardium was isolated and placed into a washing buffer solution (0.25 M sucrose in 0.025 M tris-HCl buffer with pH values of 7.5). Tissue samples were cooled and placed into Petri dishes, cut into pieces with scissors after a double or triple wash. Washing buffer was added and tissues were carefully washed. After precipitation, the fluid was carefully drained. The manipulation mentioned above was made again. Then the myocardial tissue was dried with the filter paper and homogenized in a Potter's homogenizer in 0.32 M sucrose and 0.025M of tris-HCl buffer (pH 7.5) solutions in the 1:5 ratio on ice.

The homogenates were centrifuged (600 g) during 10 minutes in a CRL-1 refrigerator centrifuge. Supernatant was used for biochemical studies.

Preparation of the *Ribes nigrum* fruit extract and selection of its dose

Ribes nigrum fruit extract was manufactured in a standard way. Extraction of anthocyanin complexes was performed using 96% ethanol. The alcohol-to-raw material ratio was 1.0 dm^3 / 100 g. The extraction lasted for 2 hours in a flask with a backflow condenser. After the extraction berries were separated from an anthocyanin alcohol solution by filtration, and the alcohol solution was evaporated using a rotary vacuum evaporator (2 revolutions at 600 °C). This method allows obtaining anthocyanins both in intact and glycosidic forms, i.e. those molecules that have a high biological activity of the original plant components (Patent UA 73974 d.d. October 10, 2012 "Process for the cumulative anthocyanins release from fruit and berries" by OD Roshal, RYu Iliashenko, VI Rosliakov).

Integral antioxidant capacity (IAC) of the antioxidant complex in the extract was determined using potentiometric method. IAC of the blackcurrant fruit extract was measured to be 81.1 mg/g.

Redox potential (ε) values of the antioxidant complex were measured by cyclic voltamperometry using an AUTOLAB PGSTAT+28N electrochemical station (USA). Measurement was performed in the standard electrochemical cell with electrodes. The carbon electrode was used as an indicator. The additional electrode was manufactured from platinum, whereas the comparative electrode was composed of Ag/AgCl. Scan velocity was 100mV/sec. Ɛ values for the antioxidant complex (phosphate buffer, pH = 7.40) were 200 and 400 mv. This indicates heterogeneity of the extract (2 redox-systems) and its ability to be reduced.

The antioxidant activity (AA) of the extract used in our research was 1.9 mg/g in terms of the equivalent amount of gallic acid. It was determined potentiometrically using a mediator system $K_3[Fe(CN)]_6/K_4[Fe(CN)]_6$ in the electrochemical cell with the Pt (EPL-02) and AgCl electrodes (EVL-1M4) using a B2-34 differential voltmeter. The current formed as a result of oxidation of molecules with reduction potential served as an analytical signal. AA = $V_A \bullet Cx \bullet V_1/V_A \bullet m\text{-}1$ (mg/g), where V_A is a sample volume taken for analysis, cm^3; Cx is a value of the antioxidant activity on the calibration curve, mg/cm^3; V_1 is a total volume of the sample analyzed, cm^3; V_1/V_A is a dilution rate; m is a mass of the sample analyzed, g.

Given the metabolic coefficient for rats, recommended daily allowance for humans and concentrations of anthocyanins in the blackcurrant extract, we calculated a daily dose of the antioxidants-containing extract for rats from group 2. We have chosen the dose of 0.1 ml/100g, because this dose of the extract has pronounced antioxidant effects.

Bioethics

All the manipulations with the animals were performed in accordance with provisions of the *European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes*. The experimental protocol was approved by the Ethical Committee of Kharkiv National Medical University (Kharkiv, Ukraine).

Detection of biochemical parameters in blood serum and myocardial homogenates

Troponin-1 and myoglobin that are known to be generally recognized markers of cardiac pathology were detected using rapid tests. Test systems from *Pharmasco* were used. Determination was performed in accordance with instructions provided by the manufacturers.

Creatine phosphokinase-MB (CPK-MB) activity was determined using *CK-MB-DAC* reagent kits (kinetic method) purchased from *DAC-Spectro-MED* (Republic of Moldova).

Total lactate dehydrogenase (LDH) activity was measured using a reagent kit manufactured by *Filicit-Diagnostika* (Dnipro, Ukraine).

The content of reduced glutathione was determined by the reaction with the Elman reagent spectrophotometrically using a CP-46 spectrophotometer (Fairbanks and Klee, 1986).

Determination of the content of TBA-reactive substances (TBARs) was performed using fluorimetric method (Fedorova et al, 1983).

Superoxide dismutase (SOD) activity was detected using a method offered by VA Kostyuk (Kostyuk et al, 1990). Catalase activity in myocardial homogenates was determined using the method of Chevari (Chevari et al, 1991). The method is based on the assessment of the H_2O_2 utilization rate from an incubation medium in a color reaction with ammonium molybdate. Catalase catalyzes decomposition of hydrogen peroxide to molecular oxygen and water. The enzyme activity was assessed by a decrease in H_2O_2, which was determined in a color reaction with ammonium molybdate based on the ability of hydrogen peroxide to form a stable colored complex with molybdenum salts.

ATP content in myocardial homogenates was measured spectrophotometrically (Prokhorova, 1982).

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activity was determined spectrophotometrically using reagent kits purchased from *Filicit-Diagnostika* (Dnipro, Ukraine).

Morphological investigation

After the sacrifice of animals the frontal incision of the heart was undertaken. Heart tissue samples were fixed in a 10% neutral formalin solution. Five-micron-thick sections were prepared from paraffin-embedded myocardium. They were stained with hematoxylin and eosin, gallocyanin-chromalum according to Einarson's technique.

Microscopy was performed using the *Axiostar plus* microscope (Zeiss). Using the *Videotest* application (St. Petersburg, Russian Federation), the optical density of cardiac hystiocyte cytoplasm stained with gallocyanin-chromalum was identified (Tashke, 2006).

Statistical analysis

Statistical analysis of numerical values obtained in this study was carried out using the *GraphPad Prism 5* software. To test the distribution normality, Kolmogorov-Smirnov test was used. Non-parametric Mann-Whitney U test was selected to compare two independent groups of variables. Data were represented as medians and interquartile ranges. A probability of $p<0.05$ was considered statistically significant.

RESULTS:

Levels of troponin-1 and myoglobin were found to be elevated in blood serum 20 minutes after the adrenaline intraperitoneal injection (positive test) in animals from group 1. The CPK-MB activity also increased (Table 1). The parameters mentioned above are well characterized and widely recognized biomarkers of cardiomyocyte destruction. They are released into the bloodstream when the membrane integrity is lost. Changes became more pronounced on day 1 after the adrenaline injection in rats from group 1.1. Colored strips on troponin-1 and myoglobin tests became thicker indicating their higher concentrations. In addition, the CPK-MB activity was 2.1-fold higher than in rats from the control group. Such findings are indicative of the expansion of the affected locus in myocardium. On day 3 of acute IHD development, the activity of CPK-MB in rats from group 1.2 decreased but it was still higher than in control animals. Troponin-1 and myoglobin were still detected confirming the presence of the pathologic process in myocardium (Table 1). However, the size of the damaged area was reduced.

Twenty minutes after the adrenaline intraperitoneal injection at a toxic dose against the background of the consumption of blackcurrant extract, the presence of troponin-1 and myoglobin in blood serum was under question (controversial results), whereas the CPK-MB activity was significantly elevated compared with the control group. However, it was 20% lower than in rats from group 1, indicating a smaller size of pathological locus in myocardium.

Results of troponin-1 and myoglobin tests in animals from group 2.1 were revealed to be negative, while the CPK-MB activity was still higher than in controls but noticeably and statistically significantly lower in comparison with rats from group 1.1 (Table 1).

Tab. 1.

The content of troponin-1 and myoglobin and activity of creatine phosphokinase MB in blood serum of rats with experimental acute ischemic heart disease (Me[25th%; 75th%])

Groups of rats	Troponin-1 (qualitative measurement)	Myoglobin (qualitative measurement)	Creatine phosphokinase MB nmol / sec×L
Control group (n=7)	Negative	Negative	31.69 [30.22; 32.08]
Adrenaline-induced acute ischemic heart disease (n=7) After 20 min	Positive	Positive	48.66 [47.55; 50.27] p<0.001
On day 1	Positive	Positive	67.54 [64.72; 69.12] p<0.001
On day 3	Positive	Positive	55.93 [54.12; 56.78] p<0.001
Adrenaline-induced acute ischemic heart disease treated with *Ribes nigrum* fruit extract (n=7) After 20 min	Controversial	Controversial	39.64 [37.96; 41.00] p<0.001
On day 1	Negative	Negative	48.59 [46.62; 49.00] p<0.001
On day 3	Negative	Negative	36.25 [34.81; 37.01] p>0.001

Note: p is a significance value compared with the control group

Neither troponin-1 nor myoglobin were detected in blood serum of animals with IHD consumed the blackcurrant extract (group 2.2) on day 3 of the experiment. Moreover, values of CPK-MB activity were only slightly higher compared with the control group. Such changes point to normalization of metabolism in the cardiac muscle.

There is strong evidence that oxidative stress triggers metabolic disorders in acute IHD (Cervantes Gracia et al, 2017; Vichova and Motovska, 2013). In this study, we evaluated the effects of *Ribes nigrum* fruit extract on lipid peroxidation and the state of antioxidant system in myocardium of rats. It was revealed that on day 1 after the adrenaline injection levels of TBARs were 3.7-fold higher in myocardial homogenates in group 1.1 compared with control animals (Table 2). Catalase and superoxide dismutase activity increased moderately. However, the elevation rate was significantly lower compared with that of TBARs. The content of glutathione in myocardial homogenates was 26% reduced in rats with acute IHD. Our findings suggest that adrenaline-induced acute IHD is associated with the activation of lipid peroxidation against the background of the insufficient antioxidant activity, i.e. oxidative stress development is observed. Oxidative stress may lead to significant metabolic disorders found in this study. Analysis of the activity of some enzymes in myocardial homogenates revealed a higher activity of AST and ALT in animals with experiment IHD. This fact allowed us to presume the increased protein catabolism in cardiac muscle during acute IHD (Table 3). In addition, CPK-MB and LDH activity was increased in animals from groups 1.1 and 1.2 against the background of a decrease in ATP concentration in myocardial homogenates (Table 3), which can be considered to be signs of energy deficiency and hypoxia. All metabolic changes mentioned above are specific for acute IHD.

Tab. 2.

Lipid peroxidation markers and antioxidant parameters in myocardial homogenates in experimental acute ischemic heart disease on day 1 after the adrenaline injection (Me[25th%; 75th%])

Groups of rats	TBA-reactive substances (nmol / g of protein)	Catalase (μkat / g of protein)	Superoxide dismutase (U / min×g of tissue)	Reduced glutathione (mmol / g of protein)
Control group (n=7)	1.73 [1.65; 1.81]	2.08 [2.05; 2.11]	32.48 [32.07; 32.51]	6.04 [5.87; 6.34]
Adrenaline-induced acute ischemic heart disease (n=7)	6.33 [6.24; 6.82] p<0.001	2.86 [2,78; 2.88] p<0.001	38.95 [38.77; 40.16] p<0.001	4.47 [4.10; 4.57] p<0.001
Adrenaline-induced acute ischemic heart disease treated with *Ribes nigrum* fruit extract (n=7)	3.42 [3.39; 3.64] p<0.001 p_1<0.001	3.76 [3.69; 3.82] p<0.001 p_1<0.001	46.59 [45.13; 46.80] p<0.001 p_1<0.001	7.71 [7.53; 7.95] p<0.001 p_1<0.001

Note: p is a significance value compared with the control group; p_1 is a significance value compared with group 1.1.

Tab. 3.

ATP content and activity of some enzymes in myocardial homogenates in experimental acute ischemic heart disease on day 1 after the adrenaline injection (Me[25th%; 75th%])

Groups	ATP (μmol/g of tissue)	Lactate dehydrogenase (mmol / g of protein×min)	Creatine phosphokinase (mmol / g of protein×min)	Aspartate aminotransferase (mmol / sec×g of protein)	Alanine aminotransferase (mmol / sec×g of protein)
Control group (n=7)	2.24 [2.09; 2.31]	17.58 [16.14; 17.65]	23.52 [22.51; 24.44]	45.59 [43.86; 46.25]	23.77 [22.79; 24.72]
Adrenaline-induced acute ischemic heart disease (n=7)	1.64 [1.54; 1.73] p<0.001	38.77 [37.75; 39.74] p<0.001	51.88 [50.29; 52.28] p<0.001	63.00 [62.77; 65.81] p<0.001	44.30 [42.28; 46.75] p<0.001
Adrenaline-induced acute ischemic heart disease treated with *Ribes nigrum* fruit extract (n=7)	1.97 [1.88; 2.08] p<0.01 p_1<0.001	27.09 [26.58; 27.45] p<0.01 p_1<0.01	34.40 [33.60; 35.27] p<0.001 p_1<0.001	52.27 [49.27; 53.28] p<0.001 p_1<0.001	32.61 [30.63; 33.09] p<0.001 p_1<0.001

Note: p is a significance value compared with the control group; p_1 is a significance value compared with group 1.1.

Intake of *Ribes nigrum* fruit extract by rats with acute IHD resulted in a slightly pronounced but the statistically significant increase in the content of TBARs with the simultaneous overactivation of superoxide dismutase and catalase and elevation of reduced glutathione in myocardial homogenates in group 2.1 (Table 3). Such findings can be interpreted as a minor imbalance in the prooxidant/antioxidant state. Analysis of metabolic parameters in myocardial homogenates showed a slight statistically significant

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upregulation of ALT, ASAT, CPK-MB, LDH and a decrease in ATP content in rats from group 2.1 compared with the control group (Table 3). However, it is worth mentioning that the degree of such changes was significantly lower than in untreated rats with acute IHD. Thus, metabolic disorders are expressed to a lesser extent against the background of treatment with the blackcurrant extract.

On day 3 after the adrenaline injection, concentrations of TBARs were still elevated (but to a lesser extent than on day 1) in rats from group 2.2. The activity of catalase and SOD, levels of reduced glutathione in rats from group 2.2 were slightly higher compared with group 1.2 in myocardial homogenates of rats treated from acute IHD with the antioxidants-containing blackcurrant extract (Table 4). Thus, the rate of oxidative stress decreases under the influence of treatment.

On day 3 from the adrenaline injection, levels of TBARs and reduced glutathione, the activity of SOD and catalase in rats from group 2.2 didn't differ from those of the intact animals (Table 4). Thus, our findings indicate the normalization of prooxidant/antioxidant state and reduction of oxidative stress. This determined the normalization of metabolic parameters in myocardium: the activities of all enzymes studied in this research (LDH, CPK-MB, AST, and ALT) were almost identical to their values in control animals (Table 5). ATP content was significantly higher than in untreated rats with IHD (Table 5).

Tab. 4.

Lipid peroxidation biomarkers and antioxidant indices in myocardial homogenates in experimental acute ischemic heart disease on day 3 after the adrenaline injection (Me[25th%; 75th%])

Groups	TBA-reactive substances (nmol / g of protein)	Catalase (μkat / g of protein)	Soperoxide dismutase (U / min× g of tissue)	Reduced glutathione (mmol / g of protein)
Control group (n=7)	1.73 [1.65; 1.81]	2.08 [2.05; 2.11]	32.48 [32.07; 32.51]	6.04 [5.87; 6.34]
Adrenaline-induced acute ischemic heart disease (n=7)	3.19 [3.08; 3.46] p<0.001	3.47 [3.38; 3.50] p<0.001	47.07 [45.79; 47.87] p<0.001	5.41 [5.15; 5.49] p<0.001
Adrenaline-induced acute ischemic heart disease treated with *Ribes nigrum* fruit extract (n=7)	2.11 [1.95; 2.18] p<0.001 p_1<0.001	2.34 [2.32; 2.37] p<0.001 p_1<0.001	31.72 [30.89; 32.21] p>0.05 p_1<0.001	6.31 [6.27; 6.52] p>0.05 p_1<0.001

Note: p is a significance value compared with the control group; p_1 is a significance value compared with group 1.2.

Tab. 5.

ATP concentrations and activity of some enzymes in experimental acute ischemic heart disease on day 3 after the adrenaline injection (Me[25th%; 75th%])

Groups	ATP (μmol / g of tissue)	Lactate dehydrogenase (mmol / g of protein×min)	Creatine phosphokinase (mmol / g of protein×min)	Aspartate aminotransferase (mmol / sec×g of protein)	Alanine aminotransferase (mmol / sec×g of protein)
Control group (n=7)	2.24 [2.09; 2.31]	17.58 [16.14; 17.65]	23.52 [22.51; 24.44]	45.59 [43.86; 46.25]	23.77 [22.79; 24.72]
Adrenaline-induced acute ischemic heart disease (n=7)	1.99 [1.96; 2.10] p<0.02	25.94 [25.42; 26.06] p<0.001	35.58 [34.14; 37.59] p<0.001	52.18 [49.32; 53.25] p<0.001	35.85 [34.32; 36.77] p<0.001
Adrenaline-induced acute ischemic heart disease treated with *Ribes nigrum* fruit extract (n=7)	2.26 [2.15; 2.30] p>0.05 p_1<0.01	16.87 [16.43; 17.41] p>0.05 p_1<0.001	24.38 [23.96; 24.65] p>0.05 p_1<0.001	45.65 [42.69; 46.62] p>0.05 p_1<0.001	24.59 [22.05; 25.60] p>0.05 p_1<0.001

Note: p is a significance value compared with the control group; p_1 is a significance value compared with group 1.2.

Thus, *Ribes nigrum* fruit extract administration increases antioxidant activity. This leads to downregulation of toxic free radical products, membrane stabilization, evidenced by a decrease in the content of organ-specific enzymes in blood serum, and normalization of the metabolic processes.

Histological study of cardiac tissue was performed to evaluate cardioprotective effects of blackcurrant extract. Myocardium of intact control animals looks more compact microscopically. Insignificant perivascular edema indicates a gradual decrease in blood circulation and development of interstitial edema in body tissues due to general venous congestion development. Cardiomyocytes are preserved. Their nuclei are oval and moderately euchromatic (gallocyanin-chromalum staining). Cross-striations are visualized. Glycogen granules are observed in cytosol of cardiomyocytes (PAS-reaction).

Animals with experimental acute IHD had more pronounced interstitial edema in myocardium. Small loci of necrosis with macrophagic and lymphocytic migration indicate myocardial ischemia (Fig. 1, 2).

In animals with IHD from group 2 treated with the *Ribes nigrum* fruit extract, interstitial edema is expressed less significantly than in untreated rats. The higher number of cardiomyocytes is characterized with larger euchromatic nuclei. Cytosol is stained more intensively (gallocyanin-chromalum Einarson's staining). This can be indicative of a higher RNA content in cardiomyocytes. Cardiomyocytes with preserved cross-striations are visualized. However, tiny macrophagic and lymphocytic infiltrates can be observed somewhere (Fig. 3, 4). This indicates a fewer number of necrotic loci formed in treated animals compared with the untreated ones. Thus, ischemic effects of adrenaline are highly likely less significant due to the *Ribes nigrum* fruit extract administration.

Morphometric study of cardiomyocytes' cytosol in experimental groups was performed. Einarson's staining for total nucleic acids showed an increase in this parameter in animals treated from IHD with the blackcurrant extract: group 1 – 0.050 ± 0.003 C.U. of absorbance, group 2 – 0.145 ± 0.007 C.U. of absorbance ($p < 0.001$). Thus, the content of RNA in cytosol of cardiomyocytes is higher after the *Ribes nigrum* fruit extract administration in experimental acute IHD.

Our histological findings suggest that the use of *Ribes nigrum* fruit extract as a treatment in experimental acute IHD leads to a decrease in the number and volume of necrosis loci in myocardium and to the elevation of the RNA content in cytosol of cardiac myocytes.

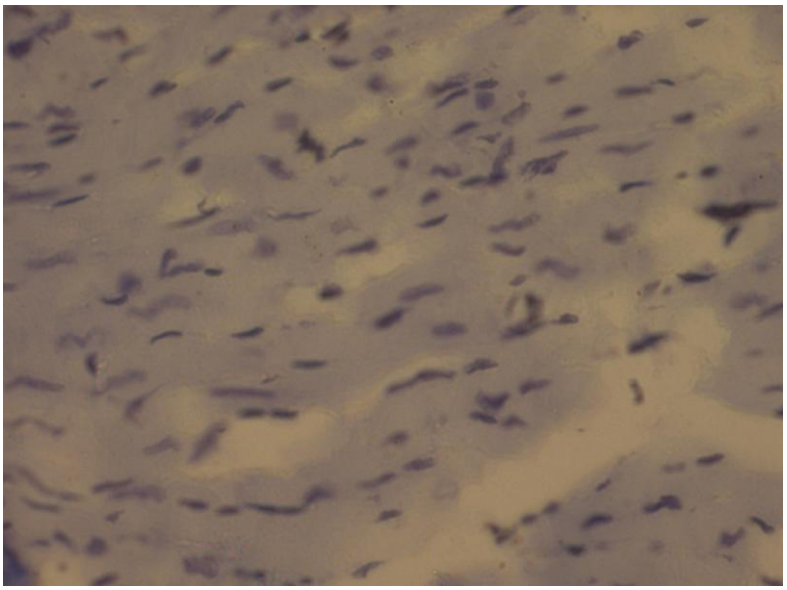

Fig. 1. Myocardium of a rat from group 1. Interstitial edema is observed. Hyperchromatic nuclei prevail in cardiomyocytes. Einarson's gallocyanin-chromalum staining. ×400.

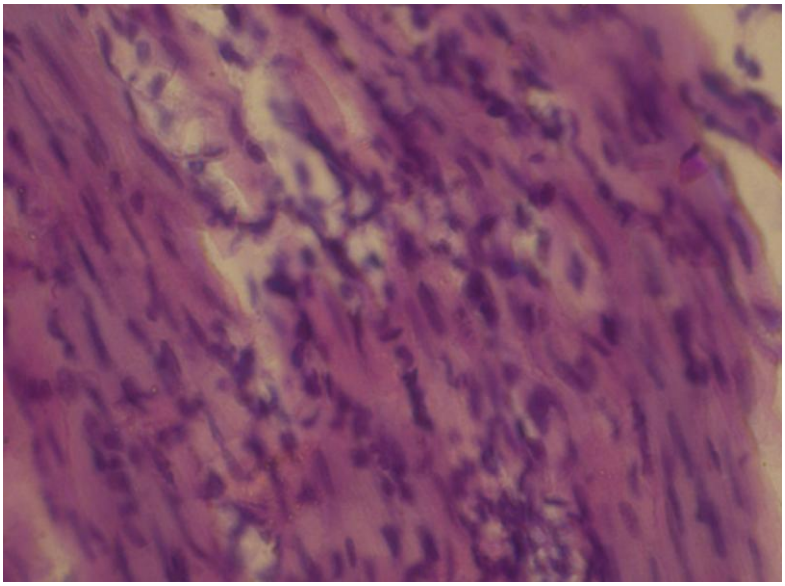

Fig. 2. Myocardium of a rat from group 1. Locus of necrosis in myocardium with signs of karyopyknosis, karyolysis, cytolysis, and leukocyte infiltration can be noticed. Hematoxylin & eosin staining. ×400.

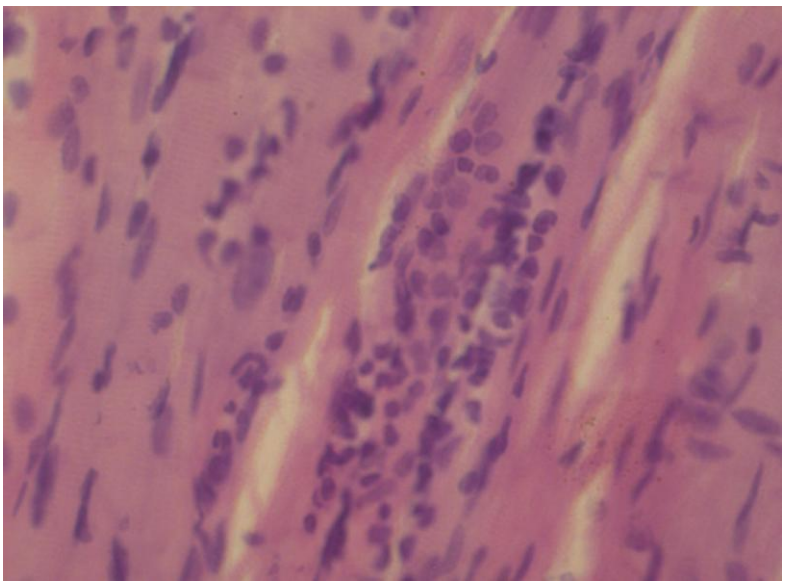

Fig. 3. Myocardium of an animal from group 2. A small locus of leukocyte infiltration is located near a small focal necrosis. Hematoxylin & eosin staining. ×400.

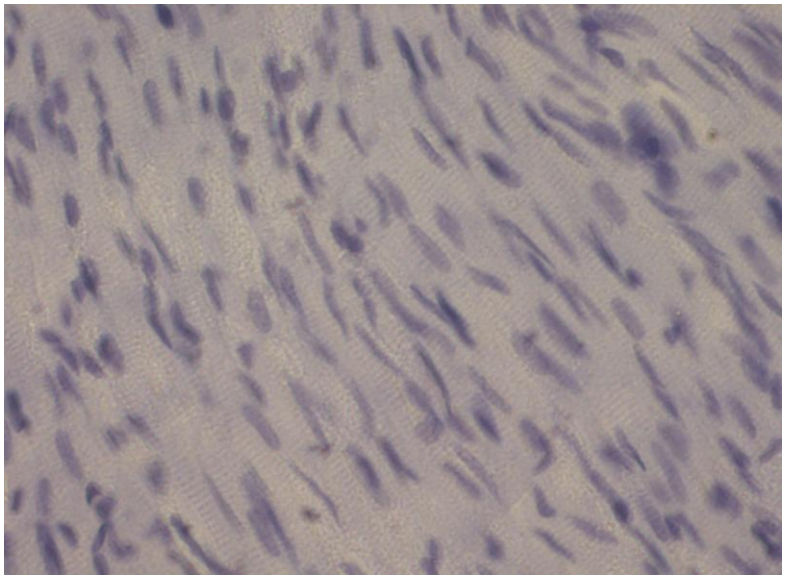

Fig. 4. Myocardium of an animal from group 2. Cross-striation of cardiomyocytes is clearly visible. Cardiomyocytes have large euchromatic nuclei. Einarson's gallocyanin-chromalum staining. ×400.

DISCUSSIONS:

Numerous studies have demonstrated that antioxidants can prevent the development of myocardial damage or restrict its manifestations. Nowadays naturally occurring antioxidants have become more popular due to their higher bioavailability and relatively lower toxicity (Pandey and Rizvi, 2009).

Among natural sources of antioxidants, bioflavonoids are extremely widespread. Rutin, quercetin, and various kinds of vitamin P extracted from citrus fruits and chokeberry are widely used. It has been known that antioxidant properties of bioflavonoids are mediated by functional hydroxyl groups (Treml and Šmejkal, 2016).

Converging lines of evidence support a high antioxidant potential of bioflavonoids. It has been demonstrated that due to their antioxidant properties bioflavonoids are able to: reduce the permeability of capillaries, improve capillary circulation, improve blood supply and transport of biologically active substances to tissues; prevent the oxidation of low density lipoproteins (LDLs) and the development of atherosclerosis (Panche et al., 2016). In particular, the antioxidant activity of bioflavonoids protects ascorbic acid from excessive oxidation, preventing the loss of its biological activity. Bioflavonoids strengthen capillary walls by reducing the hyaluronidase activity synergically with ascorbic acid, resulting in a decrease in capillary wall permeability (Zeng et al, 2015).

It has been demonstrated that bioflavonoids of plant origin affect the course of cardiovascular diseases via preventing excessive NO formation and regulation of NO bioavailability (Duarte et al 2014). It has been shown that bioflavonoids improve endothelial function in patients with cardiovascular pathology (Grassi et al, 2009).

Inflammation is known to play a pivotal role in the development of cardiovascular diseases. It is regulated by a number of biologically active substances, including cytokines, eicosanoids, etc. Some *in vitro* studies have demonstrated that bioflavonoids are able to affect inflammation-associated mediators showing anti-inflammatory effects (Leyva-López et al, 2016; Serafini et al, 2010).

Excessive platelet activation is known to promote aggregation and clot formation, which may cause thrombosis, the development of myocardial infarction and cerebrovascular disorders. Bioflavonoids have been reported to inhibit platelet aggregation (Guerrero et al, 2007).

Thus, bioflavonoids reduce cardiovascular risks and administration of naturally occurring bioflavonoids seems to be promising for the treatment of cardiovascular diseases, however, further studies are required (Peterson et al, 2012).

Among natural plant sources of antioxidants, blackcurrant, or *Ribes nigrum*, has one of the highest amounts of biologically active substances. Numerous researches focused on chemical compounds found in berries and leaves have demonstrated that blackcurrant contains: beta-carotene, vitamins A, B1, B2, B5, B6, B9, E, H PP, ascorbic acid (0.4%); sugars (4.5 – 16.8%); organic acids (2.5 – 4.5%), including citrate, malate, pectic and tannic acids; flavonoids (5-quercetin); anthocyanins (cyanidin-3-glucoside, cyanidin-3-rhamnoglucoside, delphinidin-3-rhamnoglucoside, delphinidin-3-glucoside); macroelements and microelements (Skrovankova et al, 2015).

As mentioned above, flavonoids have significant antioxidant properties. Anthocyanins from blackcurrant can strengthen the wall of the capillaries, have antioxidant, antibacterial, and anti-carcinogenic properties (Khoo et al, 2017).

Our findings support that administration of *Ribes nigrum* fruit extract in experimental acute IHD leads to a significant increase in reduced glutathione content in myocardium. SH-groups of glutathione allow it to act as a reducing agent. Its main function is to protect SH-groups in protein molecules and preserve them in the reduced state. Glutathione is also involved in decomposition of hydrogen peroxide, which is formed as a result of free radical processes Thus, an increase in glutathione concentration in myocardial homogenates of rats after the *Ribes nigrum* fruit extract administration indicated an increase in the antioxidant activity, reduced lipid peroxidation intensity, evidenced by the reduced content of TBARs in rats from group 2, and membrane stabilization, evidenced by a decrease in troponin-1 and myoglobin levels and the reduced activity of organ-specific heart-associated enzymes on day 1 after the adrenaline injection. It is worth mentioning that almost all of the antioxidants used for medical and experimental purposes increase the activity of superoxide dismutase and catalase to a greater or lesser extent. Such changes were also observed in our study.

Activation of glycolysis is observed in low-energy state (low ATP levels found in this study) and creatine phosphokinase is overactivated in oxygen deficiency (ischemia caused by the adrenaline injection). This hypothesis is confirmed by a significant increase in LDH and CPK-MB activity in myocardial homogenates of rats with experimental acute IHD on day 1 after the adrenaline injection (primarily in rats of group 1). A higher activity of LDH and CPK MB on day 3 in untreated animals compared to the treated ones might indicate the development of hypoxia in myocardium of rats with experimental acute IHD without the *Ribes nigrum* fruit extract administration and reduction of hypoxia in animals administered the blackcurrant extract.

Energy production in ischemia is one of the key factors for survival of cells. Morphological changes in myocardium differ significantly in untreated and treated rats with IHD. This may be observed due to the more pronounced activation of the antioxidant system in rats as a result of the *Ribes nigrum* fruit extract administration. This decreased manifestations of oxidative stress on day 3 after the adrenaline injection may be due to the high amount of antioxidant biologically active substances found in the blackcurrant. *Ribes nigrum* fruit extract may contribute to the activation of energy metabolism due to the presence of TCA cycle intermediates (in particular, malate) in it. Malate is known to be the TCA cycle substrate that can pass through the mitochondrial membrane in low concentrations and increase the respiratory control ratio (Maruyama et al, 2013).

Our findings suggest that the *Ribes nigrum* fruit extract administration in experimental acute IHD leads to the restriction of necrosis focus and intensified tissue repair highly likely due to the reduced rate of oxidative stress as a result of the significant activation of antioxidant system, subsequent membrane stabilization and energy metabolism normalization.

CONCLUSIONS:

Experimental acute IHD in rats is characterized by oxidative stress development, the appearance of organ-specific biochemical markers in blood serum and energy deficiency in myocardium.

Ribes nigrum fruit extract single administration at a dose of 0.1 ml / 100g of body weight results in the significant activation of both enzymatic and non-enzymatic links of antioxidant system in myocardium, a decrease in the content of TBARs on day 1 and normalization of redox metabolism on day 3 after the adrenaline injection.

Blackcurrant fruit extract administration in rats with acute adrenaline-induced IHD promotes tissue energy metabolism activation on day 1 after the adrenaline injection, normalization of ATP levels and other metabolic parameters on day 3 after the beginning of the experiment.

Ribes nigrum fruit extract is a promising agent for correction of metabolic disorders in myocardium in acute IHD.

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