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Aqueous extract of *Atriplex hortensis* and *Betulae folia* as actoprotector in rats

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Abstract:
Saltbush (*Atriplex hortensis* L.) and birch leaves (*Betulae folia*) are ubiquitous raw materials with a wide range of useful properties. This research focused on the actoprotective effect that a mixed aqueous extract of these two plants had on laboratory rats subjected to forced swim test.

The experiment included an intact group (control), rats that received treatment but underwent a forced swim test, and rats that were administered with the experimental aqueous extract of *A. hortensis* and *Betulae folia* followed by the forced swim test. The mixed extract of *A. hortensis* and *Betulae folia* (Kemerovo Region, Russia) was administered intragastrically to three-month-old male Wistar rats (4 mL/100 g body weight) who performed daily 2-h swimming sessions for two weeks.

The chemical analysis of the extract revealed the presence of flavonoids (quercetin, luteolin, kaempferol), 8 essential amino acids, and 17 amino acids, including amino acids with a branched side chain (valine, isoleucine, leucine). The forced swim test made it possible to study the effect of the extract on the hematological parameters of peripheral blood. The hematological analysis showed that the administration of the extract restored leukocytes, lymphocytes, and hemoglobin to the levels demonstrated by the animals in the intact group. As for the electrocardiographic parameters, the swimmers demonstrated a faster depolarization of the heart chambers while maintaining normal heart rate, which denoted an efficient compensation for the hypertrophic changes in the myocardium caused by the physical exertion.

In this *in-vivo* research, the extract of *Betulae folia* and aerial parts of *A. hortensis* had no cardiotoxic effect and helped restore the level of blood oxygenation after physical exertion. In the future, the synergetic actoprotective effect of these two widespread plants can be used in dietary supplements and functional foods.

Keywords: *Betulae folia*, *Atriplex hortensis* L., adaptogens, adaptive response, physical exertion, cardiovascular system, electrocardiogram, hematological analysis, rats, *in vivo*

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INTRODUCTION

The fast-paced rhythm of modern life and unfavorable environmental conditions affect human health by reducing adaptive potential and provoking stress reactions. Eventually, these two factors may contribute to the demographic pitfall by triggering the development of fatal diseases[1].

According to the World Health Organization, about 80% of deaths in the world are caused by chronic non-communicable diseases[2], especially cardiovascular disorders. The list of cardiovascular risks involves chronic stress and increased allostatic loads [1, 2]. Strenuous physical activity and psycho-emotional stressors activate the sympathoadrenal system by increasing the cardiac rate and contractility, which results in high blood

[1] World health statistics. 2023. World Health Organization Data. [cited 2025 Jul 8]. Available from: https://data.who.int/ru

[2] World health statistics...

pressure [3, 4]. While this activation has an adaptive value when caused by short-term stress, chronic stress to sympathetic nervous system, on the contrary, may have detrimental effects on the cardiovascular system [5]. Disrupted oxidative phosphorylation boosts the generation of reactive oxygen species, intensifies lipid peroxidation, and reduces energy supply to cells [6–8]. The increased blood flow rate damages the vascular endothelium [3, 4]. The high levels of stress in modern society make scientists search for effective means to promote adaptation and increase stress resilience [4, 9, 10].

Atriplex, commonly known as saltbush or orache, boasts a variety of pharmacological properties, confirmed both *in vivo* and *in vitro* [11–13]. For instance, *Atriplex halimus* L. was reported to have an antidiabetic effect [11] while *Atriplex farinosa* Forssk. and *Atriplex nummularia* Lindl. [12] demonstrated reliable antihyperglycemic, antihyperlipidemic, and antioxidant properties. *Atriplex hortensis* L. exhibited an actoprotective effect in animals subjected to cold stress [13].

Betulae folia, or birch leaves, contain an antioxidant called botulin [14, 15]. *Betulae folia* extracts were reported to exercise antibacterial effect against gram-negative bacteria *Staphylococcus aureus* [16]. They are anti-inflammatory and antinociceptive [17], hypolipidemic [18, 19] and low-toxic [17, 20].

Extracts obtained from various types of *Atriplex* plants demonstrate reliable antioxidant properties [21]. Their antibacterial and antiurease activity is associated with the high content of triterpene glycosides [22, 23].

Atriplex and *Betula* plants contain substances with a wide range of physiological activities that can be used in medicinal compositions. In this regard, the adaptogenic properties of aqueous extracts of *Betulae folia* and aerial parts of *A. hortensis* should be studied *in vivo*, i. e., on laboratory animals under physical exertion.

In this study, we examined the effect of a mixed aqueous extract of *Betulae folia* and *A. hortensis* on the adaptive response to forced physical exertion in animal models.

STUDY OBJECTS AND METHODS

Preparation. *Betulae folia* and aerial parts of *Atriplex hortensis* L. were collected near the village of Osinovka (coordinates: latitude 55°23′22″N, longitude 86°18′15″E), Kemerovo Region, Russia, in May and June, 2022 (Fig. 1).

The species were identified at the Department of Ecology and Nature Management, Kemerovo State University (Kemerovo, Russia). The plants were dried without access to direct sunlight in a ventilated room at 25 ± 2°C. Then, we ground them in a vortex mill with a classifier (TW200, Japan) to obtain fine powder with a particle size of 40–50 μm.

Physicochemical properties of plant powder. We addressed the Kjeldahl method to determine the content of crude protein in the plant powder [24]. The quantitative assessment of reducing sugars relied on the method proposed in [25]. The optical density test involved

a

b

Figure 1 Appearance of experimental plants: a – *Betulae folia*; b – *Atriplex hortensis* L.

a UV-1200 spectrophotometer (Shanghai Mapada Instruments, China); the light transmission was measured at 440 nm. To define the mass fraction of crude ash, we performed dry ashing [26] at 550–650°C in an LF-7/11-G1 muffle furnace (Russia). In the mineralized samples, we determined contents of calcium, phosphorus, iron, zinc, and copper. The amount of calcium was analyzed in line with the complexometric method while the photometric method made it possible to reveal the contents of phosphorus and iron [26]. The optical density test involved an UV-1200 spectrophotometer, which measured the light transmissions at 440–465 nm (phosphorus) and 450–490 nm (iron). The mass fraction of copper and zinc was revealed by the inversion voltammetry method. The procedure involved a voltametric analyzer AKV-07MK (Russia) with a three-electrode sensor and data collection, provided with the Aquilon processing software.

We used titration to measure total tannins [27] and high-performance thin-layer chromatography to identify and measure terpenoids (isoprenoids) and phenolics. The experiment was conducted on Sorbfil PTS-AF-A plates (Russia). The mobile phase consisted of a mix of *n*-butanol, acetic acid, and water at a ratio of 60:15:25 [28].

Antioxidant profile. Potentiometry made it possible to measure the ability of the extract to remove free radicals. This method measures the electric transient negative signal with a flow-type platinum electrode detector by the composition change in a redox-reagent solution [29].

Vitamin profile. The vitamin composition was studied using the method of high-performance liquid chromatography (HPLC) with gradient elution [30] and standard vitamin solutions (ECOS-1, Russia).

Amino acid profile. The amino acid composition was also analyzed by HPLC in an Agilent 1260 Infinity II chromatograph (Agilent Technologies, USA) equipped

with a multi-wave detector and an analytical column. After grafting the C18 carrier onto a high-purity silica gel base, we applied the gradient elution. The procedure also involved an Agilent ZORBAX Eclipse AAA device (4.6×150 mm, 3.5 μm, reversed phase). The eluents included phosphate buffer, methanol (ECOS-1, Russia), and water at a ratio of 45:45:10. The phosphate buffer was based on sodium hydrogen phosphate (Na_2HPO_4, 0.5M; Elabscience Biotechnology, China) and acetonitrile (ECOS-1, Russia). The elution rate was 2.0 mL/min; the column temperature was 40°C. The chromatograph calibration [31] relied on the following list of amino acids: aspartic acid, glutamic acid, serine, histidine, glycine, threonine, arginine, alanine, tyrosine, cysteine, valine, methionine, phenylalanine, isoleucine, leucine, lysine, and proline (Agilent Technologies, USA; Item: 5,061–3,332).

***In vivo* studies.** All manipulations with laboratory animals followed the standard recommendations issued by the International Ethics Committee. The permission to conduct the study was obtained from the Ethics Committee of the Institute of Immunology and Physiology, Ural Branch of the Russian Academy of Sciences (no. 05/23, May 24, 2023). The experiment involved 30 three-month-old male Wistar rats. The rats were kept under standard vivarium conditions with natural illumination and free access to water and food.

The rats were divided into three groups, 10 animals in each. Group 1 consisted of intact animals. Group 2 included rats that received no experimental treatment but underwent a forced swim test. Group 3 consisted of rats that were administered with the experimental aqueous extract of *A. hortensis* and *Betulae folia* followed by the forced swim test.

The physical load was simulated using the method of 2-h forced swimming sessions for 14 days [32]. The water temperature was 30 ± 1°C. The amount of plant powder in the extract based on the available pharmacological data for other types of these plants (Table 1).

The mixed aqueous extract was prepared as follows. We poured 7 g of *Betulae folia* powder and 4.3 g of *A. hortensis* powder with 200 mL of hot water (85 ± 2°C). The extraction lasted for 12 h, with subsequent filtering. Group 3 rats received the extract in an amount of 4 mL/100 g body weight for 14 days. The rats in Groups 1 and 2 received an equivalent volume of drinking water.

Cardiovascular parameters. After two weeks of daily forced swimming, the cardiovascular parameters to be analyzed on animal model involved peripheral blood hematology, electrocardiography, blood pressure, heart rate, and blood oxygenation. The rats were sacrificed under anesthesia with 20 mg/kg xylazine and 10 mg/kg Zoletil-100 intramuscularly.

The electrocardiographic examination was performed using a Zoomed iE300 veterinary electrocardiograph (Zoomed, China). The animals were placed on a warm mat (37°C); electrodes were fixed on their limbs at spots lubricated with conductive gel. We used a Microlux ML-410 Vet veterinary tonometer (Microlux, Russia) to assess the heart rate and blood pressure. The blood oxygenation test involved an M3S veterinary monitor (TooTooMeditech, China).

Peripheral blood was sampled from the tail vein in the morning after a 12-h fast. The samples were added into test tubes with K3-EDTA anticoagulant. The parameters were assessed using a veterinary hematology analyzer MindrayBC-2800Vet (Mindray, China). The list of parameters to be assessed was as follows: absolute granulocyte count (Gran, 10^3/μL); relative granulocyte count (Gran, %); hemoglobin content (Hb, g/L); hematocrit (Hct, %); absolute lymphocyte count (Lymph, 10^3/μL); relative lymphocyte count (Lymph, %); average hemoglobin content in an erythrocyte (MCH, pg); mean corpuscular hemoglobin concentration (MCHC, g/L); mean corpuscular volume (MCV, fl); absolute monocyte count (Mon, 10^3/μL); relative monocyte count (Mon, %); mean platelet volume (MPV, fl); platelet crit (PCT, %); platelet size distribution (PDW, %); platelet content (PLT, 10^3/μL); absolute erythrocyte count (RBC, 10^6/μL); erythrocyte size distribution (RDW, %); and absolute leukocyte count, (WBC, 10^3/μL).

Statistical analysis. The primary processing was carried out in the MS Excel 365 program. The data were presented as the arithmetic mean (m) ± standard error (SD). To test the hypothesis of homogeneity of two independent samples, we used the nonparametric Mann-Whitney U test and the nonparametric Wilcoxon test for dependent samples. When testing the statistical hypotheses, we applied a 5% significance level. The calculations were performed using the Origin 201 software.

RESULTS AND DISCUSSION

Figure 2 and Tables 2–5 illustrate the chemical components in *Atriplex hortensis* L. and *Betulae folia* that affected the rats' metabolism under physical loads.

Table 1 Justification of plant powder dose in the extract

Plant species	Amount	Property tested	Reference
Atriplex halimus L.	200 mg/kg body weight	Antidiabetic	[11]
Atriplex farinosa Forssk., *Atriplex nummularia* Lindl.	200–400 mg/kg body weight	Antihyperglycemic, antihyperlipidemic, antioxidant	[12]
Atriplex hortensis L.	2 mL/100 g body weight	Actoprotective (in animals under induced cold stress)	[13]
Betula platyphylla Sukacz.	400 mg/kg body weight	Anti-inflammatory, anti-nociceptive	[17]
Betula pendula Roth	1.5 g/100 g diet	Hypolipidemic (polysaccharides)	[18]
Betula pendula L., leaves	3 g/100 g diet	Hypolypidemic (polysaccharides)	[20]

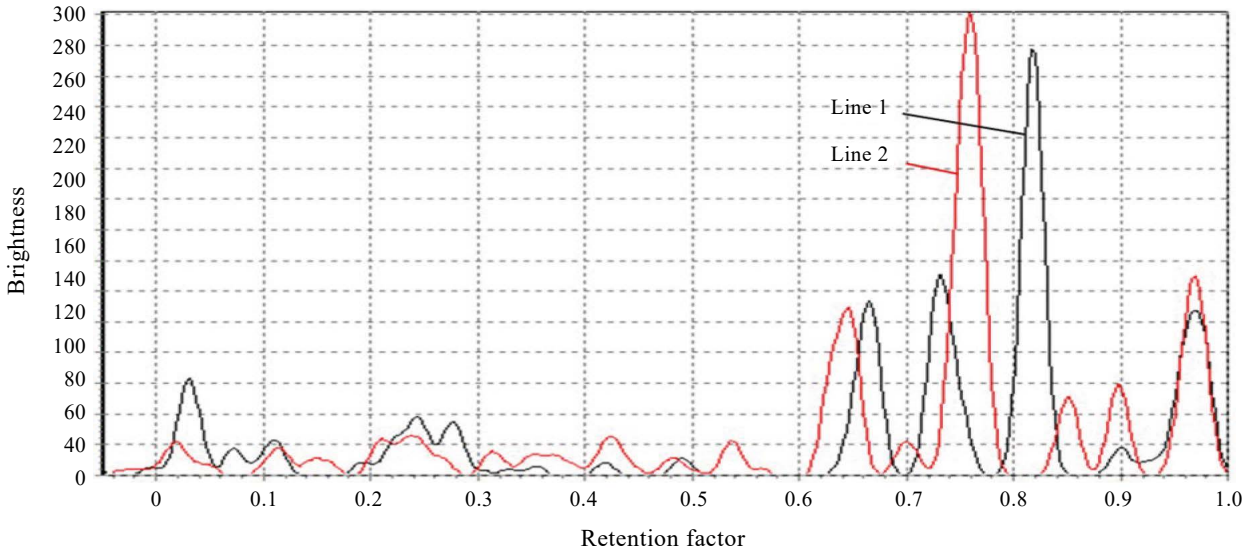


Figure 2 Densitogram of aqueous extracts: Line 1 – *Atriplex hortensis* L.; Line 2 – *Betulae folia*

Table 2 Physicochemical analysis of *Atriplex hortensis* L. and *Betulae folia* (m ± SD)

Constituents	*Atriplex hortensis* L.	*Betulae folia*
Mass fraction, %:		
crude protein	30.45 ± 0.61	9.69 ± 0.65
reducing sugars	0.86 ± 0.02	0.61 ± 0.02
tannins	2.74 ± 0.08	1.04 ± 0.05
flavonoids	1.10 ± 0.02	1.60 ± 0.03
ash	18.24 ± 0.36	1.27 ± 0.36
calcium	2.43 ± 0.06	0.27 ± 0.04
phosphorus	0.371 ± 0.009	0.140 ± 0.002
iron	0.0157 ± 0.0003	–
Contents, mg/kg:		
copper	0.442 ± 0.011	4.11 ± 0.08
zinc	6.73 ± 0.13	34.15 ± 0.70

Table 3 Vitamin composition of *Atriplex hortensis* L. and *Betulae folia* (m ± SD)

Constituents	Contents, mg/100 g	
	Atriplex hortensis L.	*Betulae folia*
Ascorbic acid (C)	98.6 ± 1.9	56.9 ± 0.9
Pantothenic acid (B_5)	105.0 ± 2.1	30.7 ± 0.5
Pyridoxine (B_6)	9.31 ± 0.20	0.80 ± 0.02
Nicotinamide (B_3)	3.20 ± 0.11	0.30 ± 0.01
Thiamine (B_1)	4.40 ± 0.08	0.80 ± 0.02
Folic acid (B_9)	1.10 ± 0.02	0.70 ± 0.01

The aerial parts of *A. hortensis* proved to be rich in crude protein (Table 2), as well as in vitamins C, B_5, and B_6 (Table 3).

Intense physical activity exhausts metabolic processes, inducing an increased need for vitamins B, PP, C, etc. Vitamins, especially group B, and their derivatives are involved in the biosynthesis of enzymes responsible for biochemical reactions associated with the oxidation of nutrients and energy production. Their consumption has to be increased to inhibit the lipid peroxidation processes, which get stronger under heavy psychophysical stress. These processes trigger the need in antioxidant vitamins, in particular, vitamin C [33].

Calcium is one of the valuable constituents of *A. hortensis* Physical exercise may decrease the levels of circulating serum calcium while increasing the levels of parathyroid hormone and bone resorption. These disturbances in calcium and bone metabolism can be reduced by taking calcium supplements immediately before exercises [34].

Iron, copper, selenium, cobalt, manganese, and zinc are essential microelements affected by physical exercise [35]. *Betulae folia* contained significantly more copper and zinc than *A. hortensis* If consumed as supplements, these mineral components may restore the metabolic and microelement status of the body during physical exercise. The modulation of zinc and selenium homeostasis is especially important [35].

The high-performance liquid chromatography detected 17 amino acids in the plant extracts under study. *A. hortensis* had 26,115 mg of total amino acids while *Betulae folia* contained as little as 4,793 mg/100 g body weight. The *Amaranthaceae* plants of genus *Atriplex* contain record amounts of protein and amino acids, depending on the species [13, 36–40]. *Betulae folia* had the same amino acids as *A. hortensis*, but in much lesser quantities. However, the combination of these plants can enhance the biological effect. Figure 3 illustrates the data obtained on the ratio of amino acids, color red marking particularly high percentages.

The amino acid profile of *A. hortensis* differed significantly from that of *Betulae folia* in such amino acids as alanine, cystine, lysine, and proline. Their proportion in the total amino acids for *A. hortensis* was 2–3 times higher than in *Betulae folia*. Both plants demonstrated very similar balance of essential amino acids, except

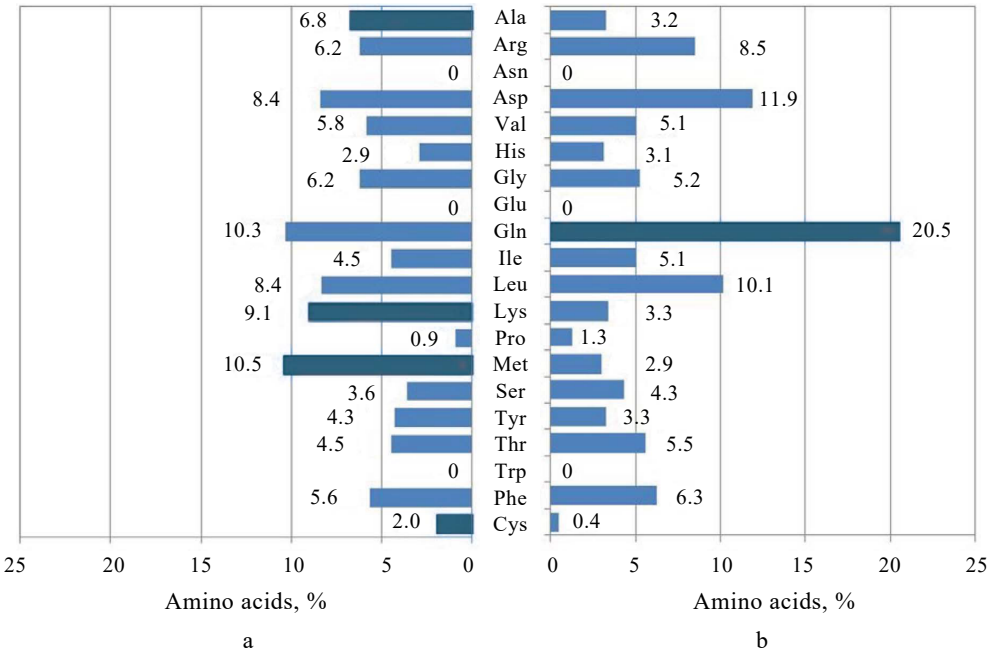


Figure 3 Amino acids in *Atriplex hortensis* L. (a) and *Betulae folia* (b)

for lysine: its proportion in *A. hortensis* was three times as high as in *Betulae folia*. However, *Betulae folia* contained twice as much glutamic acid as *A. hortensis* Glutamic acid is not essential, but it plays a very important role in nitrogen metabolism. The species and geographic location seemed to have no significant effect on the vitamin and amino acid composition of the studied plants. Our research results were consistent with publications regarding *Betulae folia* [41, 42] and other species of *Atriplex* from different regions of the world [39, 43, 44].

The antioxidant activity of the aqueous plant extracts was determined by the potentiometric method: 0.260 ± 0.005 mmol-Eq/L for the extract of *Betulae folia* and 0.271 ± 0.005 mmol-Eq/L for the extract of *A. hortensis*. The high antioxidant capacity means that the plant extracts remove free radicals, rendering protection from various stresses and physical exertion [45].

Flavonoids are antioxidants that can be consumed with food; they are important components of the cellular antioxidant system [46–52] and provide antimicrobial protection [53]. Flavonoids owe their antioxidant properties to their ability to trap free radicals and chelate metal ions involved in peroxidation [54, 55]. Polyphenolic compounds (Phen) are able to interact with hydroxyl (L-O•)-radicals and peroxyl (L-OO•)-radicals of lipids (alkoxyls) due to their ability to donate an electron (or a hydrogen atom). This phenomenon leads to the formation of phenol radicals (phenoxyls, which do not participate in oxidation). Their molecule has a unique structure associated with the electron cloud stabilization [56, 57]:

$$\text{L-OO}^{\bullet} + \text{Phen-OH} \rightarrow \text{L-OOH} + \text{Phen-O}^{\bullet}$$
$$\text{L-O}^{\bullet} + \text{Phen-OH} \rightarrow \text{L-OH} + \text{Phen-O}^{\bullet}$$

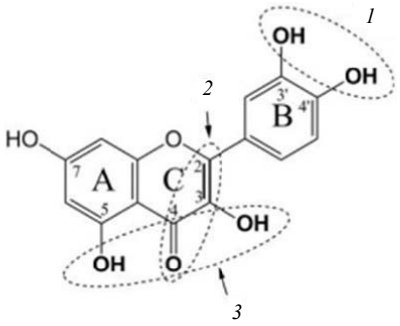


Figure 4 Quercetin molecule

Flavonoid molecules have three regions responsible for the binding of free radicals: (*1*) a group of two adjacent hydroxyls on the *B*-ring called the catechol group; (*2*) a 2,3-double bond conjugated with a 4-oxo group, which presumably initiates the delocalization of *B*-ring electrons; and (*3*) hydroxyl groups in positions 3 and 5, which capture radicals (Fig. 4) [46].

Oxidants usually target the hydroxyls of the catechol group of the *B*-ring or the hydroxyl group at the C-3 position. When these groups are oxidized, short-living semiquinone anion radicals are formed, followed by orthoquinones [58, 59]. Depending on the number and location of hydroxyl groups, flavonoids have the highest activity in the presence of the diphenylpicrylhydrazine radical [46]: quercetin → luteolin → kaempferol. Both *A. hortensis* and *Betulae folia* contain these highly active flavonoids (Fig. 3, Table 3).

Currently, no general theory links the structure of flavonoids with their antioxidant activity, but it can be assumed that flavonoids have different specializations in protecting the body from various damaging agents.

Table 4 Hematological parameters across rat groups

Blood parameters	Group 1 (intact)	Group 2 (water + swim test)	Group 3 (extract + swim test)
Absolute leukocyte count, 10^3/μL	7.68 ± 0.40	5.63 ± 0.69[a]	5.95 ± 0.64
Absolute lymphocyte count, 10^3/μL	5.16 ± 0.32	3.38 ± 0.37[a]	4.39 ± 0.48
Absolute monocyte count, 10^3/μL	0.40 ± 0.03	0.20 ± 0.04[a]	0.25 ± 0.04
Absolute granulocyte count, 10^3/μL	2.12 ± 0.08	2.05 ± 0.60	1.31 ± 0.21[a]
Relative lymphocyte count, %	66.94 ± 0.85	61.40 ± 6.37	73.79 ± 2.59
Relative monocyte count, %	5.06 ± 0.50	3.93 ± 0.44	4.38 ± 0.61
Relative granulocyte count, %	28.02 ± 0.72	34.68 ± 6.68	21.83 ± 2.32[a, b]
Absolute erythrocyte count, 10^6/μL	10.02 ± 0.14	9.67 ± 0.23	9.94 ± 0.15
Hemoglobin content, g/L	160.80 ± 2.18	147.25 ± 3.59[a]	156.00 ± 1.22
Hematocrit, %	55.94 ± 0.73	53.28 ± 1.48	55.45 ± 0.69
Mean corpuscular volume, fl	55.90 ± 1.32	55.20 ± 1.17	55.85 ± 0.71
Average hemoglobin content in an erythrocyte, pg	16.02 ± 0.36	15.20 ± 0.33	15.64 ± 0.19
Mean corpuscular hemoglobin concentration, g/L	287.20 ± 1.50	276.00 ± 1.08[a]	280.90 ± 1.93[a]
Erythrocyte size distribution, %	12.38 ± 0.37	12.08 ± 0.26	12.17 ± 0.16
Platelet content, 10^3/μL	1,319.24 ± 62.66	1,003.75 ± 38.25[a]	1,660.40 ± 44.56[a, b]
Mean platelet volume, fl	6.04 ± 0.04	6.20 ± 0.15	6.03 ± 0.07
Platelet size distribution, %	15.68 ± 0.04	16.03 ± 0.13	15.89 ± 0.07[a]
Platelet crit, %	0.80 ± 0.03	0.62 ± 0.03[a]	0.60 ± 0.01[a]

[a] – significant difference with Group 1 ($p < 0.05$); [b] – significant difference with Group 2 ($p < 0.05$)

Figure 5 Forced swim test

To claim that some flavonoids are more effective than others, we would have to take into account the specific experimental conditions and the structural features of free radicals. A wide variety of flavonoids in nature allows these substances to provide a comprehensive protection of organisms from many different environmental threats. As in the case of vitamin and amino acid composition, the species and geographic location had no effect on the antioxidant capacity index [40, 60–62].

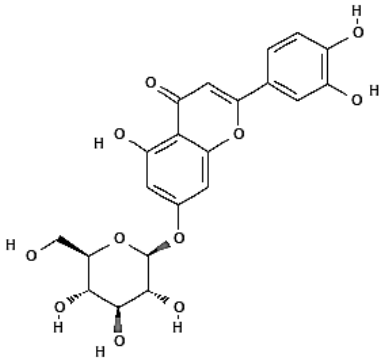
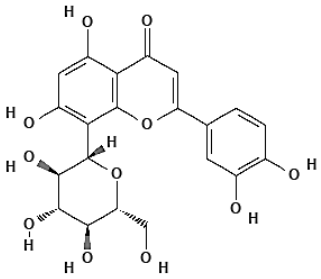
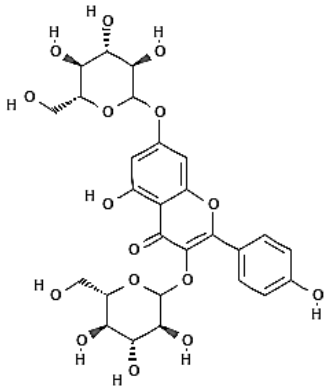
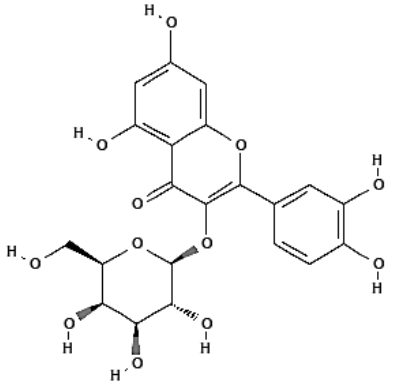
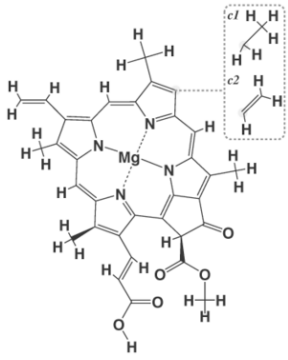
Effect of mixed aqueous extract on peripheral blood hematology in rats after forced swim test. Physical and stress loads, in synergy or separately, are known to have a significant effect on hematological indices [4]. The forced swim test (Fig. 5) took place daily in two-hour sessions for two weeks, thus leading to the third stage of stress. It reduced the total leukocyte content in the blood of the test rats, affecting the lymphocyte and monocytic lineages. Table 5 also visualizes the lowering hemoglobin content in red blood cells, as well as the average concentration of hemoglobin in red blood cells, platelets, and platelet crit.

Different hematopoietic lineages have different sensitivities to stress hormones. Lymphopenia can apparently be associated with the activation of the sympathoadrenal system and the inhibitory effect of glucocorticoids on lymphopoiesis. For the erythrocyte and platelet lineages, the effect of glucocorticoids usually manifests itself in hyperplastic phenomena, which we did not detect in this research. On the contrary, we detected a response decrease in the platelet count, which was consistent with other publications on the effect of repeated physical activity [32]. A slight decrease in hemoglobin could be a sign of anemia [63], indicating a lack of plastic resources for adequate hematopoiesis under conditions of strenuous physical activity [64]. However, this indicator for Group 3 (experimental) approached the indicator for Group 1 (intact) due to the additional supply of iron from the combined aqueous plant extract.

The intragastric administration of the extract to the experimental rats restored the total content of leukocytes, lymphocytes, and hemoglobin to the level recorded in the intact animals (Table 4). Both the plants contained branched-chain amino acids, i. e., leucine, isoleucine, and valine (Table 5[3]). They are known to boost immune resistance and adaptive potential during physical exertion [65, 66]. The platelet content in the

[3] Quickly find chemical information. PubChem. [cited 2025 Jul 8]. Available from: https://pubchem.ncbi.nlm.nih.gov

Table 5 Flavonoids and pigments in *Atriplex hortensis* L. and *Betulae folia*

Retention factor	Substance	Structural formula
0.65–0.67	Luteolin-7-glucoside	
0.73–0.76	Luteolin-8-glucoside	
0.82–0.85	Kaempferol 3,7-di-O-glucoside	
0.90	Quercetin-3-o-galactoside	
0.97	Chlorophyll	

experimental group was higher than in the intact rats (Group 1) and those rats that received water instead of the plant extract. This fact requires a more detailed study since it might indicate an increase in the thrombogenic potential while the functional aggregation activity is undoubtedly important [67]. The low content of various types of leukocytes in the blood is a typical reaction of the blood system to a high-intensity physical activity [68]. At the stage of urgent adaptation, the counts of neutrophils, monocytes, and lymphocytes go down. At the stage of long-term adaptation, leukocytes restore to the level of various classes in the following order: neutrophils, monocytes, and lymphocytes [69]. In our research, the aqueous extract affected the sequence of restoration of leukocytes in the experimental rats (Group 3).

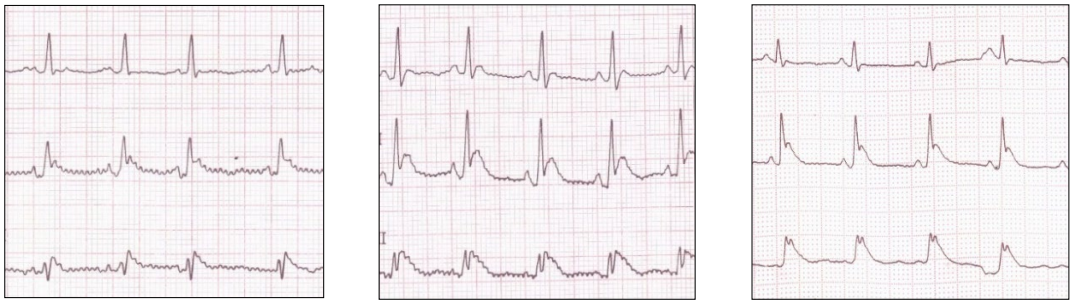
Effect of mixed aqueous extract on cardiovascular system in rats after forced swim test. For Group 2 (water + swim test) and Group 1 (intact), the two-week forced swim test for 2 h daily led to a slight decrease in the blood pressure and blood oxygenation by 8 and 10%, respectively (Table 6). Apparently, the short-term loads used in the experiment caused no sharp breakdown of the adaptive mechanisms in the cardiovascular system. However, the blood pressure and heart rate in the experimental rats in Group 3 (extract + swim test) decreased by 15 and 18%, respectively, with a simultaneous increase in the saturation level to the indicator of the intact animals (Group 1). This fact further contributed to the development of a long-term adaptive mechanism by preventing injury to the vascular endothelium as a result of intensified blood flow [3, 4]. We may conclude, however, that the experimental extract had no cardiotoxic effect in the applied amount.

Effect of mixed aqueous extract on electrocardiogram parameters in rats after forced swim test. We performed an electrocardiographic analysis to assess the effect of the experimental plant extract on the cardiac activity of the rats during physical exertion (Fig. 6, Table 7).

Table 6 Cardiovascular system parameters across rat groups

Cardiac indicators	Group 1 (intact)	Group 2 (water + swim test)	Group 3 (extract + swim test)
Systolic blood pressure, mm Hg	166.80 ± 9.66	153.00 ± 10.00[a]	142.00 ± 8.95[a, b]
Diastolic blood pressure, mm Hg	101.60 ± 5.23	93.00 ± 5.73[a]	85.20 ± 5.81[a, b]
Blood oxygenation, %	87.20 ± 2.63	79.00 ± 2.12[a]	89.80 ± 3.26
Heart rate, bpm	181.60 ± 9.18	176.75 ± 8.00	149.80 ± 15.03[a, b]

[a] – significant difference with Group 1 ($p < 0.05$); [b] – significant differences with Group 2 ($p < 0.05$)



a b c

Figure 6 Electrocardiograms across groups: a – Group 1 (intact); b – Group 2 (water + swim test); and c – Group 3 (extract + swim test)

Table 7 Electrocardiogram parameters across groups

Electrocardiogram	Group 1 (intact)	Group 2 (water + swim test)	Group 3 (extract + swim test)
P-wave height	1.18 ± 0.08	1.50 ± 0.20	0.85 ± 0.09[a, b]
P-wave time	1.56 ± 0.12	1.38 ± 0.08	1.13 ± 0.10[a]
R-wave height	8.60 ± 0.87	9.38 ± 1.43	6.63 ± 0.58
R-wave time	1.46 ± 0.10	1.43 ± 0.14	1.08 ± 0.04[a, b]
QRS time	6.20 ± 0.20	6.88 ± 0.31	4.38 ± 0.80
R-R interval	15.40 ± 1.48	14.88 ± 0.77	15.00 ± 0.91
Height of T-wave (II lead)	2.84 ± 0.55	6.00 ± 0.35[a]	3.88 ± 0.84
T-wave time (II lead)	2.40 ± 0.24	3.38 ± 0.31	2.88 ± 0.10

[a] – significant difference with Group 1 ($p < 0.05$); [b] – significant difference with Group 2 ($p < 0.05$)

The electrocardiogram demonstrated no significant differences in the heart rate and R-R intervals with a stable sinus rhythm, which indicated the absence of significant changes in the cardiac conduction system (Fig. 6, Table 7). The 2-h daily swimming sessions for 14 days doubled the amplitude of the T-wave, and a 40% longer T-wave time was a tendency. These changes, together with the overall slight increase in the P-waves, R-waves, and the QRS complex, indicated the development of compensatory (physiological) left ventricular myocardial hypertrophy in response to the increased physical load [70].

The experimental animals (Group 3), which received the mixed aqueous extract of *A. hortensis* and *Betulae folia*, demonstrated a lower heart rate and no change in R–R intervals in comparison with the swimmers that received no experimental treatment and with the intact animals (Fig. 6, Tables 6 and 7). The trends included a significant decrease in the amplitude of the P-wave (by 43%) and the R-wave time by (by 24%), as well as a shorter QRS complex (by 36%). Compared to the intact animals, the height and time of the P-wave went down by 28 and 27%, respectively. Also, the R-wave time decreased by 26% relative to the physiological norm.

The main differences in the electrocardiogram parameters in the experimental rats that received the extract consisted in a more rapid depolarization of the heart chambers while maintaining the heart rate, which led to longer relaxation periods, i. e., a longer rest time for the myocardium [13]. These differences may indicate a compensation for the hypertrophic changes in the myocardium caused by the physical exertion. The compensation mechanism may consist in a change in the electrolyte composition of blood plasma [71], which leads to a more rapid depolarization of all heart chambers but a slower repolarization than in the control group.

CONCLUSION

The intragastric administration of the aqueous extract of *Betulae folia* and aerial parts of *Atriplex hortensis* L. restored the total leukocytes, lymphocytes, and hemoglobin in the rats after two weeks of daily forced swim test. The experimental extract obviously demonstrated good prospects as an actoprotector. In addition, the extract affected the sequence of restoration of leukocytes during physical exertion. The test animals also showed a faster restoration of hematological parameters and hypertrophic compensation in the myocardium induced by physical exertion.

The obtained results indicate that the mixed aqueous extract of birch leaves and saltbush may become part of a an actoprotective supplement.

CONTRIBUTION

All authors contributed to the study conception and design. I.Yu. Sergeeva and T.F. Kiseleva developed the research concept; I.Yu. Sergeeva and A.V. Anshukov designed the research methodology; A.S. Markov was responsible for the software; L.V. Permyakova and Y. Huang provided validation; V.V. Atuchin performed the formal analysis; A.V. Anshukov and E.A. Mukhlynina conducted the experiments; Y. Li wrote the review; S.Yu. Kleymenov provided data curation; L.A. Ishchuk and E.A. Mukhlynina drafted the manuscript; I.Yu. Sergeeva reviewed and proofread the manuscript; A.S. Markov was responsible for the visualization; A.V. Anshukov and I.Yu. Sergeeva supervised the project; V.V. Atuchin provided funding acquisition. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

CONFLICT OF INTEREST

The authors declared no potential conflict of interest regarding the publication of this article.

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