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ANTIOXIDANT ACTIVITY OF A ADAPTOGENIC PLANT REMEDY IN MODEL SYSTEMS *IN VITRO*

ABSTRACT

The antioxidant activity of the dry extract from the complex plant remedy has been studied in model systems *in vitro*. The complex plant remedy includes the following species of medicinal plants: *Serratula centauroides* (L.), *Bergenia crassifolia* (L.) Fritsch, *Rosa davurica* Pall., *Inula helenium* L., *Echinacea purpurea* (L.) Moench. It has been established that the plant remedy under study inhibits the oxidation of the biological substrate preventing from the destruction of β – carotene (IC_{50}=24.3 μg/ml) and yolk lipoproteids (IC_{50}=65.8 μg/ml) and having membrane stabilizing effect in peroxide (IC_{50}=0.97 μg/ml) and osmotic hemolysis (IC_{50}=0.11μg/ml) of erythrocytes. The complex remedy manifests the radical binding activity for 2,2-diphenyl-1- picrylhydrazyl (DPPH$^{\cdot}$) (IC_{50}=61.4μg/ml), superoxide anion-radical (IC_{50}=28.6 μg/ml), nitrogen oxide (IC_{50}=55.3 μg/ml), also for Fe^{2+} (IC_{50}=639.3 μg/ml). The marked antioxidant activity of the tested remedy is due to the complex of biologically active substances (flavonoids, tannins, polyphenolic compounds, phenol carbonic acids, ecdysteroids, etc.) contained in its components.

Keywords: plant remedy, adaptogens, *Serratula centauroides* (L.), *Bergenia crassifolia* (L.) Fritsch, *Rosa davurica* Pall., *Inula helenium* L., *Echinacea purpurea* (L.) Moench., membrane stabilizing activity, 2.2-diphenyl-1- picrylhydrazyl, superoxide anion-radical, nitrogen oxide.

Introduction. Adaptation providing the body balance control in response to the changes in the external and internal environment is one of the main properties of living beings. The decline in adaptive skills or overstrain of adaptation mechanisms caused by pathogenic affects results in the development of pathological states due to the dysregulation of adaptive mechanisms manifested as so named "civilization diseases". Among dysmetabolic and desadaptative pathologies are: chronic heart insufficiency, cerebral circulatory insufficiency and especially the combination of the heart and brain vessel pathologies, dysfunction of central and peripheral neuroendocrine systems [17; 16]. To increase the resistance of the body to adverse health impact the various groups of medicinal means are used; among them adaptogens having the wide spectrum of pharmacological effects and increasing the resistance of the body to adverse exposure are commonly used. Their capability to regulate the hormone-mediated influence and lipid peroxidation plays a leading role in the mechanism of their effect [15; 19].

In this connection, it seems advisable the use of adaptogens of plant origin which have a systemic action on the body manifesting antioxidant, anti-inflammatory, psychotropic, cardio-protective and other effects due to the synergism of biologically active substances [6; 7; 17; 18; 19].

At the Institute of General and Experimental Biology SB RAS the dry extract has been derived from the complex plant remedy consisting of the aerial part of *Serratula centauroides* (L.), leaves of *Bergenia crassifolia* (L.) Fritsch, *Rosa davurica* Pall., *Inula helenium* L., *Echinacea purpurea* (L.) Moench. Previous experiments have shown that the given complex plant remedy has the marked actoprotective activity increasing physical endurance in rats due to optimization of the energy metabolism [2], as well it manifests anti-stress and anti-depressive effects in chronic stress [9].

The aim of the study is to estimate the antioxidant activity of the adaptogenic plant remedy in model systems *in vitro*.

Material and methods of investigation. The subject of the study was the complex plant remedy in the form of the dry extract consisting of *Serratula centauroides* (L.), *Bergenia crassifolia* (L.) Fritsch, *Rosa davurica* Pall., *Inula helenium* L., *Echinacea purpurea* (L.) Moench.

The method of the dry extract obtaining involves the extraction of the powdered plant material by 30-70% ethyl alcohol, concentration and drying in the vacuum set [12].

The membrane stabilizing activity of the tested remedy was estimated in the models of peroxide and osmotic hemolysis with the 1% erythrocyte suspension (Er/m). The peroxide hemolysis of erythrocytes was induced by Fenton reagent [22] and osmotic hemolysis – by adding of the distilled water into the incubation medium [4]. The tested plant remedy was studied in 0.01; 0.1; 1.0; 10; 100 and 500µg/ml concentrations. Ascorbic acid (*Sigma Aldrich, USA*) was used as a preparation of comparison in final concentrations: 0.0002; 0.001; 0.01; and 0.5 µg/ml. The specimens were analyzed in the spectrophotometer at the 540 nm wavelength. The membrane stabilizing effect of the tested remedy and the preparation of comparison was estimated in percentage terms in regard to the indices in the control (incubation medium free of the dry extract).

The antioxidant activity of the tested remedy was estimated according to the level of its influence on the dynamics of peroxide destruction of β-carotene (PDBC) in the DMSO-H_2O_2-oleic acid system [15]. The influence of the plant extract on the process of protein metal-catalyzed modification was studied in the model biological system (MBS) of yolk lipoproteids (YLP) [8]. The antiradical activity was estimated in regard to the stable radical 2.2-diphenyl-1- picrylhydrazyl (DPPH˙) [14]; in regard to superoxide radicals (O_2) in the nonenzymatic system phenazine methosulfate/NADN [21]; also in regard to NO molecules [21]. Fe^{2+}-chelating activity of the dry extract was determined with the use of o-phenanthroline method [13]. Quercetin, rutin, arbutin and ascorbic acid were used as substances of comparison (*Sigma Aldrich, USA*). All *in vitro* experiments were carried out in three replications. The means of the data obtained were expressed in terms of concentration required for binding 50% reactive particles in the incubation medium (IC_{50}). The data obtained were processed statistically according to recommendations [3].

Results and discussion. The research has shown that the dry extract of the complex plant remedy has the marked membrane stabilizing activity *in vitro* experiments (Table 1). Inclusion of the tested plant extract into the incubation medium decreased the intensity of OH-mediated oxidation of the erythrocyte plasma membrane. The concentration of the extract in the incubation medium up to 500µg/ml was followed by increasingly membrane stabilizing activity. Further strengthening of the tested remedy resulted in the plasma membrane rupture and denaturation of hemoprotein (the data are not presented). The concentration of the dry extract inducing 50% inhibition of the peroxide erythrocyte hemolysis process was 0.97µg/ml. Besides, the complex remedy when interacting with the erythrocyte plasma membrane promoted its permeability in hypotonic conditions resulting in the decrease of osmotic hemolysis intensity (IC_{50} = 0,11µg/ml). The revealed action of the tested plant remedy is due to the membrane stabilizing effect of the plant components presenting in its content: *S. centauroides, B. crassifolia, R. davurica* which are rich in polyphenolic complex, tannins and flavonoids having the membrane stabilizing effect [5; 10].

The data given in the Table 2 have shown that the complex plant remedy has the marked antioxidant properties preventing β-carotene from the peroxide destruction (IC_{50} = 24.3µg/ml) and inhibiting the degradation of lipoproteins in metal-catalyzed oxidation (IC_{50} = 65.8µg/ml). The activity of the tested remedy in the given model systems was comparable with the substance of comparison – arbutin.

It has been established that the dry extract has the marked antiradical effect (Table 3). The tested remedy manifests activity in respect of DPPH molecules that is due to the presence of phenolic compounds in its content. The value of DPPH 50% binding by the plant remedy was 61.4 µg/ml which surpassed the same index for the substance of comparison – arbutin.

The experiments for the evaluation of the tested remedy capacity to bind the active forms of oxygen (O_2 and NO) and mixed valence metals have revealed its antiradical activity in regard to the given reactive particles. The complex plant remedy manifests the marked Fe^{2+}-chelating activity (IC_{50}=639.3 µg/ml) which is higher than in quercetin, rutin and arbutin (Table 3).

The tested plant remedy interacts with O_2-radical more intensively in the *in vitro* model system than ascorbic acid and arbutin (IC_{50} = 28.6µg/ml). O_2-binding activity of the complex remedy is comparable with quercetin (IC_{50} = 31.2µg/ml). *In vitro* study has shown that the tested plant remedy manifests the marked activity in regard to NO molecules binding (IC_{50} = 55.3 µg/ml). The plant remedy surpasses quercetin and ascorbic acid trailing only the arbutin in this kind of activity.

Thus, the data obtained have demonstrated that the complex plant remedy has the marked antioxidant effect. The given activity is obviously due to the pronounced antioxidative activity of its components: *B. crassifolia* (hydroquinone, arbutin, dihydroquercetin, quercetin, rutin, gallic acid) [10]; *R. davurica* (quercetin, hyperoside, hyperin, gallic acid) [10]; *I. helenium* (quercetin, caffeic acid, scopoletin, umbelliferone) [11].The components of the complex remedy contain biologically

Table 1

Membrane stabilizing activity of the dry extract from the adaptogenic plant remedy in the model system *in vitro*

Subject	Concentration, µg/ml	Peroxide hemolysis, %	Osmotic hemolysis, %
Dry extract	500	11,25±0,33	6,70±0,14
	100	11,01±0,41	10,21±0,51
	10	36,91±1,24	28,60±1,62
	1	40,82±1,15	37,82±2,11
	0,1	49,27±3,10	50,23±2,45
	0,01	74,47±2,55	58,54±2,05
	IC50, мкг/мл	0,97±0,02	0,11±0,01
Ascorbic acid	0,5	27,89±1,12	10,57±0,43
	0,1	34,85±1,20	14,38±0,35
	0,01	48,86±2,31	24,80±1,10
	0,001	56,56±2,17	50,60±2,02
	0,0002	67,70±2,42	55,49±2,18
	IC50, мкг/мл	0,009±0,0003	0,002±0,0001

Table 2

Antioxidant activity of the dry extract from the adaptogenic plant remedy, IC_{50}

Subject	PDβC, µg/ml	MBS-YLP, µg/ml
Dry extract	24,3±2,11	65,8±3,17
Quercetin	10,2±0,29	16,2±1,34
Rutin	9,7±1,11	18,8±1,23
Arbutin	25,2±1,23	73,1±3,73
Ascorbic acid	9,1±0,42	39,3±2,12

Table 3

Antiradical activity of the dry extract from the adaptogenic plant remedy, IC_{50}

Subject	Reactive molecules			
	DPPH˙, µg/ml	$O_2^{\cdot-}$, µg/ml	Fe^{2+}, µg/ml	NO, µg/ml
Dry extract	61,4±2,13	28,6±3,10	639,3±11,15	55,3±2,31
Quercetin	10,3±0,54	31,2±2,11	>5000	170,2±4,21
Rutin	15,1±1,13	2,3±0,15	>5000	15,3±1,12
Arbutin	105,4±2,71	>550	>5000	35,7±1,26
Ascorbic acid	4,8±0,15	91,3±3,22	110±6,42	975,0±27,13

active substances (flavonoids, tannins, polyphenolic compounds, phenol carbonic acids, ecdysteroids, etc) having the property to form phenoxy radicals, chelate mixed valence metal ions, bind OH and O_2 molecules it promotes stabilization and structural-functional reintegration of the plasma membrane [1].

In this connection the further study of the antioxidant activity of the given complex plant remedy *in vivo* experiments seems to be advisable.

Conclusion. 1. The dry extract of the adaptogenic plant remedy promotes preservation of the structural-functional integrity of the erythrocyte membrane in plasma membrane OH-mediated oxidation and osmotic injury *in vitro*;

2. The tested plant remedy manifests the marked antioxidant activity preventing from biomacromolecule oxidation in model systems;

3. The dry extract demonstrates the antiradical activity *in vitro* in regard to such reactive particles as 2,2-diphenyl-1-picrylhydrazyl, superoxide anion-radical, nitrogen oxide and Fe^{2+} ions.

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MORPHOFUNCTIONAL INDICATORS OF ORGANISM OF THE ATHLETES-WRESTLERS OF YAKUTIA

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ABSTRACT

The article is devoted to a comprehensive study of the physical development and physique of freestyle wrestlers, as well as functional parameters of the body. The indicators of cardiovascular system - dynamics of indicators of adaptive potential and coefficient of endurance of athletes in different seasons of the year are studied.

38 men of Yakut nationality, aged 18 to 29 years, athletes – freestyle wrestlers having high sports qualification were the **object** of our study. The comparison group was 20 male cadets of the police school. The compared groups were comparable in age. The study was conducted in different seasons: summer (June), autumn (October), winter (December), spring (March).

We revealed that, among highly qualified wrestlers Yakut nationality, dominated the brachymorphic somatotype, characterized by an average or low growth, relatively long torso, broad shoulders, a large breast, short lower limbs. Analysis of the data showed that 34.2% of the athletes surveyed by us were overweight, as well as high values of the Rohrer index. Low heart rate values are probably a sign of adaptation to intense physical activity. The increase in AP points indicates signs of CVS stress, which is associated with an increase in physical and psycho-emotional stress in the autumn due to the beginning of the annual cycle of training, and in winter and spring with participation in competitions of various levels. The increase in EC (> 16.e.) 10% -18% of the surveyed us freestyle wrestlers indicates the voltage of the myocardium, and decrease in EC (< 12 e.) 45% -55% may be a sign of exhaustion of the myocardium.

Keywords: athletes, cardiovascular system, blood pressure, adaptive potential, coefficient of endurance.

Adaptation is one of the basic concepts in physiology. In the Far North, the adaptation of the human body to the conditions of habitat in high latitudes takes a special place and is provided by the restructuring of all body systems. The harsh climate also affects anthropometric indicators. T.I. Alekseeva [1], describing the «Arctic» adaptive type, indicates such features of the physical constitution of the indigenous population as a small body length, relatively wide chest, muscular body type, high body density. G.K. Stepanova [12] notes that the comparative analysis of the study of the dynamics of anthropometric data for 20 years showed that the growth in the population of young Yakuts significantly increased, but was not accompanied by the addition of body weight. This is consistent with the data of S.P. Permyakova [5] and V.G. Starostin [8] who noted an increase in the dolichomorphy among the indigenous peoples of the North and a decrease in the prevalence of representatives of the brachymorphic somatotype over the same period of time.

The human circulatory system is responsible for the adaptation of the body to various environmental factors. In most cases, the cardiovascular system (CVS) can be considered as an indicator of the body's adaptation. The study of CVS reactions allows to measure the level of functioning of the circulatory system, such as minute and shock blood volume, pulse rate, blood pressure, as well as to calculate such integrative indicators as the adaptive potential (AP) and endurance coefficient (EC) of CVS. Under excessive physical exertion in extreme Northern conditions, there are adaptive changes in athletes CVS, change in hemodynamic parameters. Adaptation to physical activity is accompanied by an increase in the impulse of the heart. The increase in the impulse of the heart affects the pulse at rest, it becomes much less frequent [6, 9 -12].

The aim of the research was to study the morphometric parameters of physical development and evaluation of the functional state of the body of freestyle wrestlers of Yakutia.

Materials and methods of research. The object of our study were 38 men of Yakut nationality, aged 18 to 29 years, athletes – freestyle wrestlers of School of the highest sports skill of Yakutsk and students of the Institute of physical culture and sports M.K. Ammosov NEFU having high sports qualification: candidates for masters of sports, masters of sports, masters of sports of international class, honored masters of sports. The comparison group was composed from 20 male cadets of the Yakutsk police school attending classes in general physical training. The compared groups