

Changes in the State of Cell Membranes of an Animal Organism Against the background of the Protective Effect of Antioxidants Under Conditions of High Temperature

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Abstract—The morphological and functional condition of biological membranes of cells and their resistance while exposed to high temperature of 41⁰ C for 180 minutes were studied in this research. The damaging effect of exogenous hyperthermic pressure was corrected by the preliminary administration of antioxidants unitiol and α -tocopherol. Malondialdehyde in blood serum was researched as the content of the final product of lipid peroxidation. Indicators of blood cell resistance of experimental animals were evaluated. The osmotic resistance of leukocytes and erythrocytes, the indicators of permeability of erythrocyte membranes, the level of peroxide hemolysis of red blood cells, the number and speed of urea hemolysis of red blood cells were studied. It was discovered that acute experimental hyperthermia causes activation of the process of formation of peroxide radicals. Significant changes occur in the processes associated with the compensation of free radical oxidation. The preliminary injections of antioxidant agents - unitiol and α -tocopherol - given to experimental animals, reduces the negative outcomes of the damaging effects of hyperthermia and has a pronounced normalizing effect.

Keywords—*hyperthermia, erythrocytes, leucocytes, lipid peroxidation, antioxidants.*

I. INTRODUCTION

The study of issues related to solving the problem of change in the resistance of the organism is aimed at identifying effective ways to increase the resistance of cells, tissues and the whole organism to damage.

Influences of the environment on the behavior of biological membranes are necessary living conditions [1]. The problem of exposure of the organism to high temperature continues to be relevant despite already conducted studies in this area [2]. The versatile influence of the thermal factor on the resistance of the organism is established [3,4].

Exposure to high temperatures leads to the development of hyperthermia, which is usually accompanied by a number of biological and physicochemical changes that affect the normal course of vital functions of the organism and lead to profound functional disorders, up to the development of heat shock and death [5,6].

In this regard, studies of the patterns, nature and characteristics of the development of cell reactivity to extreme temperatures are one of the urgent tasks of modern biology and medicine [7].

In addition, it was discovered that the resistance of the whole organism to various damaging effects is largely determined by the level of adaptive capabilities and resistance of its cells and tissues [8].

Modern biomedical research has allowed to form the notion that the most important factor in the development of various pathological processes in the organism is the stimulation of reactions of lipid peroxidation [9,10].

Blood cells participate in the progress of specific and nonspecific reactions. Thus the study of the morphofunctional characteristics of blood cells under the influence of extreme factors on the organism is of particular interest [11].

It was established that the initiation and deployment of lipid peroxidation reactions is accompanied by damage to the membrane structures of cells [9, 12].

It is known that biomembranes play an important role in cell vital activities. In this regard, damage to membrane cell structures is considered as the most important key element in the pathogenesis of many pathological conditions [11,13,14], including thermal damage.

II. RESEARCH METHODOLOGY

Based on the important role of lipid peroxidation processes in the occurrence of the effects of high temperature, it becomes clear that using substances with an antioxidant effect is appropriate to protect the organism from the damaging effects of heat.

A. Purpose of the study

To reveal the morpho-functional status of biological cell membranes and to study the resistance of animal cells exposed to thermal effects. To investigate the possibility and effectiveness of correction of changes done by antioxidants

unitiol and α -tocopherol that occur under the impact of high temperature.

B. Objectives of the study

1) To determine the effect of high temperature on the content of the final product of lipid peroxidation - malondialdehyde in the blood serum of experimental animals.

2) To study the resistance indicators of red blood cells and white blood cells of experimental animals subjected to overheating.

3) To research the effect of antioxidant substances on the nature of structural and metabolic changes and cell resistance under conditions of overheating.

C. Research methodology

Experimental studies were carried out on 400 white rats weighing 150-180 grams and 200 white mice weighing 14 - 20 grams. The animals used in the experiment were divided into experimental and control (intact) groups, they were kept in the same vivarium conditions on a normal diet and water.

The experimental animals were subjected to acute overheating, that was caused by incubation in a heat-ventilated chamber at a temperature of + 41°C for three hours.

The rectal temperature of the animals under these temperature conditions ranged from + 39°C to + 41°C.

15 animals were used in each experimental group.

All the studied parameters were determined against the background of overheating and preliminary protective action of unitiol and α -tocopherol.

Experimental and control animals of different series we studied for:

- the level of malondialdehyde (MDA) of the blood serum was determined by the content of thiobarbiturate - a dependent MDA product in the blood serum of experimental animals based on the method of Uchiyama M. и Michara M. [15, 16]

The main blood cells - leukocytes and erythrocytes, in which changes in the following parameters were researched, were also studied for:

- osmotic resistance of erythrocytes [17].;
- erythrocyte membrane permeability [14];
- level of erythrocyte peroxide hemolysis [18];
- blood red blood cell count [19];
- speed of erythrocyte urea hemolysis [20];

- osmotic resistance of leukocytes [21,22].

Vitamin E was introduced intramuscularly as a tread substance in the form of a 1% oil solution at a dose of 10 mg / kg body weight, unitiol - intraperitoneally in the form of a 5% solution at a dose of 400 mg / kg once a half hour before exposure in a heat chamber on the day of the experiment. Statistical analysis was performed using parametric methods of analysis. The arithmetic mean and its error ($M \pm m$), standard deviation (δ) were determined using the Microsoft Excel - 2003 spreadsheet editor. The reliability of differences in the average values of the indicators was evaluated using t-criterion of Student. Differences were considered reliable at significance level $p \leq 0,05$.

III. RESULTS

A research was made of a change in the indicator of lipid peroxidation (LPO) processes - malondialdehyde in the blood serum of animals under the influence of high temperature on the organism under the use of α -tocopherol and unitiol as antioxidants.

The results of the experiments show (Table I и Fig. 1) that with endogenous hyperthermic effects on the organism of experimental animals, the level of MDA in blood serum increases significantly by 32%.

The injection of antioxidant unitiol to experimental animals 30 minutes before exposure to the heat chamber leads to some normalization, while the MDA level was 107.6%.

As seen in Fig. 1 and Table I the administration of α -tocopherol before overheating of the animal also leads to a reliable normalization of the damage caused, in this case the MDA level increases by 2.3% compared with the control group (Table I).

Thus, in accordance with the data obtained, it was established that acute experimental hyperthermia has a damaging effect on blood cells of rats and causes activation of the emergence of peroxide radicals. The use of antioxidant drugs before the activation of the damaging factor is accompanied by varying degrees of pronounced normalization of the level of malondialdehyde.

Table II and in Fig. 2 present the data on the nature of changes in some indicators of resistance of blood cells to the impact of exogenous high-temperature pressure on the animal organism under the preliminary use of antioxidants unitiol and α -tocopherol. During overheating of experimental animals, changes in blood resistance indicators were the following. A significant increase in the rate of erythrocyte hemolysis was determined (by 34.5%) compared with the control group of experimental animals.

TABLE I. INFLUENCE OF ANTIOXIDANTS ON MDA LEVEL IN RAT BLOOD SERIUM ($M \pm m$)

Indicators of blood serum	Animal group			
	Control	Overheating	Unitiol + Overheating	α -tocopherol + Overheating
MDA (mk mol / l)	1.71 ± 0.04	2.26 ± 0.09 ^a	1.84 ± 0.03 ^a	1.76 ± 0.01

^a. reliable at $p < 0,05$

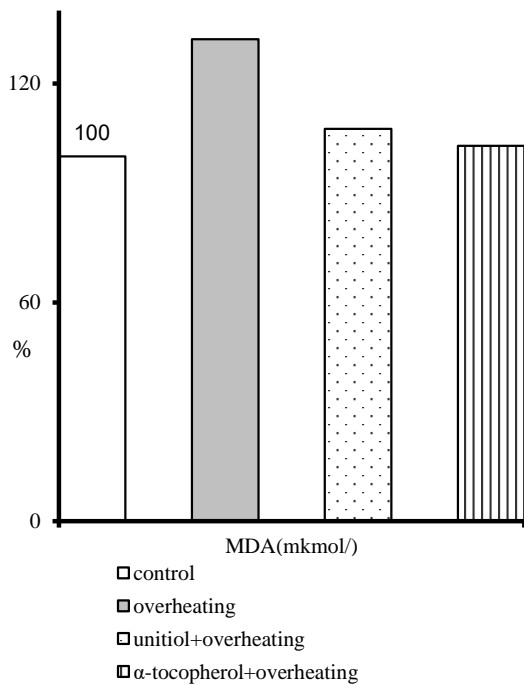


Fig. 1. The effect of antioxidants on the level of MDA in blood serum (in % against the control)

The number of red blood cells increased by 27.3% and the level of red blood cell peroxidation increased by 105.4%.

The studied parameters underwent changes presented in Table II и Fig. 2 against the background of the preliminary administration of unitiol and α-tocopherol to animals subjected to hyperthermia.

The introduction of the unitiol antioxidant that preceded overheating was accompanied by normalization of the arising changes of the studied parameters and amounted to 124.4% of the rate of hemolysis of erythrocytes, 119.7% of their number and 122.3% of the level of peroxide hemolysis relative to the control group. The membrane permeability index was also adjusted against the background of preliminary administration of unitiol.

The administration of α-tocopherol to rats before exposure to high temperature also caused a normalization of the changes of the studied parameters, with the hemolysis rate being 106.3%, the number of red blood cells - 124.7%, the level of peroxide hemolysis - 133.7%, and the erythrocyte membrane permeability rate - 128.5% accordingly, in relation to the indicators of the control group of experimental animals.

Table II and Fig. 2 also present data on the nature of changes in the permeability of erythrocyte membranes. According to the data, when overheating, there is a significant increase (by 66%) in the permeability index.

TABLE II. INFLUENCE OF ANTIOXIDANTS ON RESISTANCE OF ANIMAL BLOOD CELLS SUBJECTED TO OVERHEATING (M±M)

Indicators of resistance	Animal group			
	Control	Overheating	Unitiol + Overheating	α-tocopherol + Overheating
Red blood cell hemolysis rate (sec.)	119.96 ± 6.29	161.30 ± 15.80 ^a	149.27 ± 8.32 ^a	127.50 ± 3.04
Erythrocyte count (· 10 ⁶ in mm ³)	5.5957 ± 0.2025	7.1216 ± 0.3349 ^a	6.6970 ± 0.2279 ^a	6.9780 ± 0.3300 ^a
Red blood cell hemolysis (%)	3.32 ± 0.34	6.82 ± 0.34 ^a	4.06 ± 0.24	4.44 ± 0.31 ^a
Penetration rate of the membranes of the erythr. (rel. to un.)	6937.0 ± 315.0	11518.0 ± 283.0 ^a	9144.0 ± 412.0 ^a	8916.0 ± 397.0 ^a

^a reliable at p < 0,05

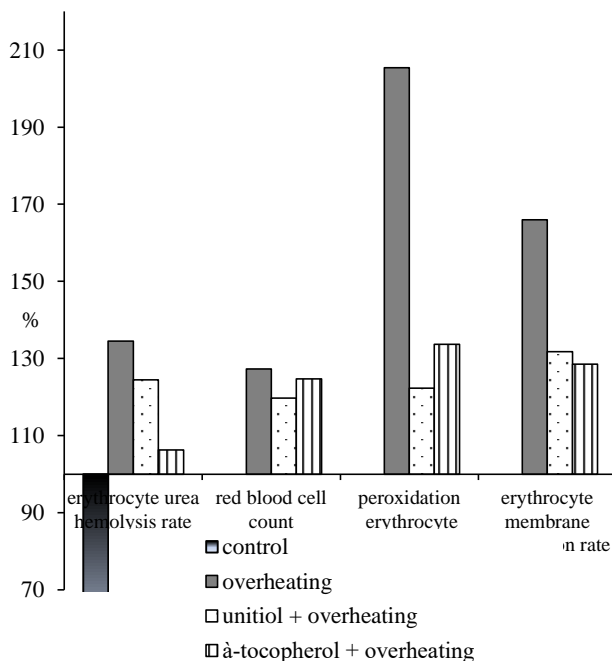


Fig. 2. Changes in indicators of resistance of blood cells against the background of the use of antioxidants during overheating (in % to control group)

The administration of antioxidants also favorably affects the value of this indicator. Against the background of unitiol injection, an increase in the permeability index is observed only by 31.8%, and after administration of α-tocopherol - by 28.5% compared with the control group.

The data received during the experiment indicate that a high-temperature effect causes a decrease in the resistance of blood cells, whereas the administration of antioxidants unitiol and α-tocopherol before the heating plays a protective role and leads to the defense of membrane structures due to the normalization of their permeability.

Table III, Fig. 3 and Fig. 4 illustrate the change in the degree of hemolysis of red blood cells of white rats in isotonic solutions of urea (18 g / l) and sodium chloride (8.5 g / l) after preliminary overheating of the animal, and the use of antioxidant preparations.

In the study process, it was found that the osmotic resistance of leukocyte and erythrocyte cells of animals that were subjected to overheating undergoes changes. It was discovered that under the impact of high temperature there is a significant increase in the degree of hemolysis of erythrocytes in rat blood, in sodium chloride solutions and urea solutions of different concentrations. At the same time, there is an increase in the degree of level of hemolysis for the

ratios of isotonic solutions of urea and sodium chloride 40:60 (by 20.5%), 45:55 (by 14.5%), 50:50 (by 6.7%), 55: 45 (by 8.7%), 60:40 (by 21.0%), 65:35 (by 15.2%).

The data obtained in the study of the effect of antioxidants on the researched processes indicate that unithiol and α -tocopherol stabilize the degree of hemolysis for all the studied mixtures.

As can be seen from the data presented in Table III, Fig. 3 and Fig. 4, administration of unithiol to rats before exposure to a heat chamber causes an increase in the degree of hemolysis in mixtures of working solutions 40:60, 45:55, 50:50, 55:45, 60:40, 65:35 (by 4.66%; 3, 4%; 5.5%; 5.9%; 10.5%; 6.7%).

The preliminary administration of α -tocopherol also causes stabilization of the level of hemolysis of erythrocytes in relation to the control group in mixtures of the same working solutions by 10.0%; 5.9%; 5.0%; 5.4%; 6.4%; 12.9%, respectively.

The administration of antioxidants to rats led to the most significant normalization of the degree of hemolysis in the 40:60 mixture, where the greatest increase was observed upon overheating. The minimum stabilization of the indicator was observed in a 50:50 mixture of working solutions.

Thus, the data of the studies performed allowed us to establish that overheating of the body leads to an increase in the degree of erythrocyte hemolysis in isotonic solutions of urea and sodium chloride of various ratios, and the administration of unithiol and α -tocopherol antioxidants to laboratory animals before animals are placed in the heat chamber normalizes the shifts that occur;

Table IV and Fig. 5 illustrate the change in the osmotic resistance of leukocytes of animals after exogenous high-temperature exposure and against the background of the previous administration of antioxidants.

The results obtained in the experiment data show that overheating causes a significant increase in the number of blood leukocytes.

TABLE III. THE DEGREE OF HEMOLYSIS OF ERYTHROCYTES IN MIXTURES OF ISOTONIC SOLUTIONS OF UREA (18G/L) AND SODIUM CHLORIDE (8.5 G/L) OF DIFFERENT CONCENTRATIONS WHEN HEATING AND ON THE BACKGROUND OF THE PREVIOUS ADMINISTRATION OF ANTIOXIDANTS (IN %)

Working solutions of urea and sodium chloride	Animal groups			
	Control	Overheating	Unithiol + Overheating	α -tocopherol + Overheating
40+60	80.84 ± 2.05	97.42 ± 5.31 ^a	84.61 ± 0.80	88.95 ± 0.93 ^a
45+55	82.94 ± 2.29	94.96 ± 3.88 ^a	85.80 ± 1.40 ^a	87.84 ± 0.50 ^a
50+50	89.07 ± 2.03	95.05 ± 1.63 ^a	93.95 ± 1.02 ^a	94.31 ± 1.38 ^a
55+45	86.93 ± 1.30	94.54 ± 1.22 ^a	92.11 ± 0.20 ^a	91.64 ± 1.86
60+40	88.80 ± 2.30	107.49 ± 7.05 ^a	98.10 ± 2.10 ^a	94.47 ± 1.15 ^a
65+35	88.81 ± 2.41	102.34 ± 3.97 ^a	94.81 ± 0.85 ^a	100.26 ± 1.29 ^a

^a reliable at $p \leq 0,05$

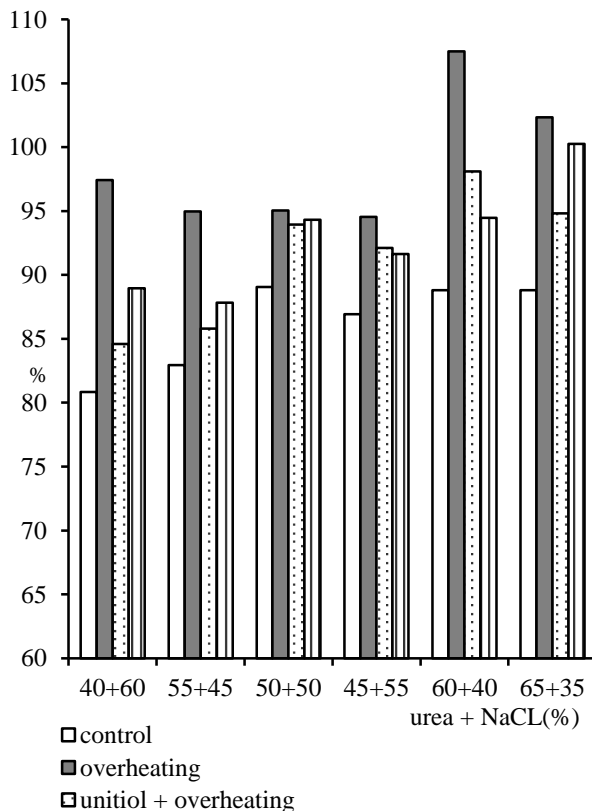


Fig. 3. Effect of antioxidants on the osmotic resistance of erythrocytes to overheating background (% to control group)

In addition, 46% increase in the number of leukocytes in hyperthermic animals was established in relation to the control group at the time of blood sampling.

During the experiment, every 30 minutes after the first count of the number of leukocytes, a change in the number of test cells was observed. It was determined that overheating leads to a decrease in the osmotic resistance of leukocytes, while the number of surviving cells was 82% after 30 minutes, 70% after 60 minutes, 58% after 120 minutes and 41% after 180 minutes. The prophylactic administration of unithiol and α -tocopherol antioxidants before exposure to high temperature to animals is characterized by less significant changes in the percentage of preserved leukocytes than when overheated.

Administration of unithiol to rats before incubation in a heat ventilated chamber led to a less significant decrease in the osmotic resistance of leukocytes, while the percentage of surviving cells was 90% after 30 minutes, 71% after 60 minutes, 58% after 120 minutes, 49% after 180 minutes.

The introduction of α -tocopherol preceding overheating also caused a change in the osmotic resistance of blood leukocytes. After 30 minutes, 92% of leukocytes remained intact, after 60 minutes 77%, after 120 minutes 60%, after 180 minutes 50%.

We can conclude that high temperature causes a significant increase in the number of blood leukocytes and a decrease in the osmotic resistance of leukocytes. The

administration of the antioxidants unithiol and α -tocopherol preceding overheating had a normalizing effect on the nature of the resulting changes and was expressed in an increase in

the osmotic resistance of blood leukocytes, with α -tocopherol having the most significant protective effect.

TABLE IV. CHANGES IN THE OSMOTIC RESISTANCE OF LEUKOCYTES IN ANIMAL BLOOD WHEN OVERHEATED ($M \pm m$)

Animal groups	Leukocyte count (10^3 in 1mm^3) preserved during research after				
	0 min.	30 min.	60 min.	120 min.	180 min.
Control	7.92 \pm 0.46	7.34 \pm 0.38	6.46 \pm 0.12	5.01 \pm 0.15	4.28 \pm 0.08
Overheating	11.56 \pm 0.48 ^a	9.54 \pm 0.48 ^a	8.13 \pm 0.42 ^a	6.66 \pm 0.33 ^a	4.74 \pm 0.16 ^a
Unithiol + Overheating	11.27 \pm 0.56 ^a	8.36 \pm 0.32 ^a	6.9 \pm 0.03 ^a	5.38 \pm 0.08 ^a	4.55 \pm 0.09 ^a
α -tocopherol + Overheating	10.92 \pm 0.27 ^a	8.26 \pm 0.05 ^a	7.08 \pm 0.10 ^a	5.40 \pm 0.11 ^a	4.48 \pm 0.08 ^a

^a reliable at $p < 0,05$

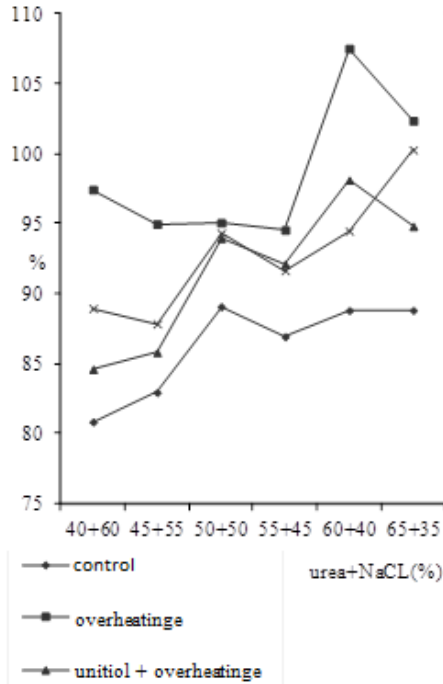


Fig. 4. The osmotic resistance of erythrocytes in rat blood upon overheating and against the background of preliminary administration of antioxidants (in %)

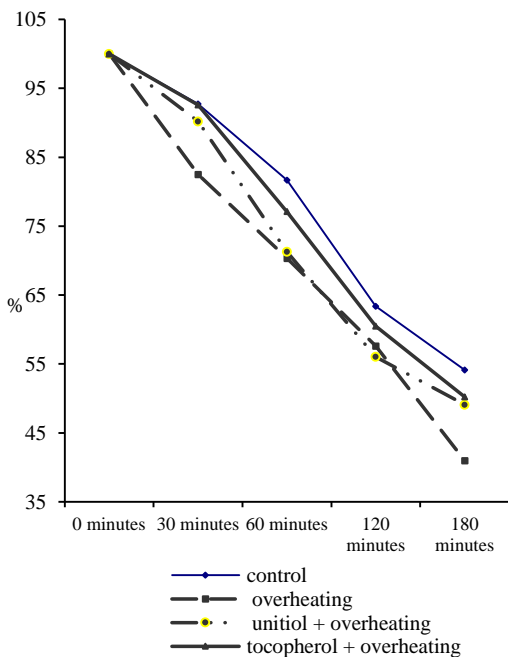


Fig. 5. Dynamics of osmotic resistance of leukocytes during overheating and against the background of the use of antioxidants (in % of control)

IV. CONCLUSIONS

The data obtained as a result of the study suggest that lipid peroxidation is involved in compensatory and pathological reactions developing in animal organism under extreme temperature conditions and determines the degree of arising pathology.

It was discovered that the cells of the animal organism react in a special way to thermal damage; in particular, significant changes occur in processes closely associated with the compensation of free radical oxidation.

In accordance with the obtained experimental data, the following conclusions can be drawn:

1. The effect of high temperature on the organism of white rats causes the activation of the processes of free radical oxidation of biosubstrates. Such a violation is expressed by an increase in the level of the content of the final product of lipid peroxidation - MDA.

2. Overheating of the animal organism leads to a decrease in the osmotic resistance of blood leukocytes. This manifestation of thermopathology can be considered as a result of a violation of the permeability of cell membranes due to the activation of SRO processes.

3. Exposure of the organism to high temperature (+ 40°C for 3 hours) causes a significant decrease in the resistance of blood cells, accompanied by an increase in the number of erythrocytes. an increase in the rate and degree of their osmotic hemolysis, as well as an increase in the level of peroxide hemolysis of red blood cells. At the same time, the permeability of the erythrocyte membrane increases. The revealed changes indicate that overheating is accompanied by changes of the morphological and functional state of erythrocyte membranes associated with their damage.

4. The administration of antioxidant drugs - unithiol and α -tocopherol - to experimental animals before overheating reduces the negative effects of the damaging impact of hyperthermia and has a pronounced normalizing effect on experimental animals. The introduction of antioxidants that can reduce the level of free radicals prior to overheating consists of inhibiting the processes of lipid peroxidation, accompanied by an increase in the resistance parameters of the animal organism.

This phenomenon is apparently the result of weakening of the processes of free radical oxidation in the structure of cell membranes and the restoration of a normal level of permeability.

The obtained experimental data are the basis for the development of methods to increase the resistance of the animal organism to the impact of high temperature with the help of drugs with antioxidant effects - unitiol and α -tocopherol.

The data obtained as a result of the conducted experiments are a prerequisite for the use of antioxidant agents unitiol and α -tocopherol as drugs that normalize reactivity and increase resistance to the damaging effects of high temperature extremes.

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