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The role of plant adaptogen in the correction of parameters of the hemostasis system during emotional-immobilization stress in the experiment

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Abstract

Background. Maladaptive changes on the part of various organs and systems, in particular on the part of the hemostasis system, are manifested in extreme forms of physical inactivity, one of which is immobilization stress. If the organism is also subjected to psycho-emotional effects at the same time, then the combined effect of stressors is accompanied by an increase in maladaptive disorders.

Aim. To establish the influence of emotional-immobilization stress on the hemostasis system, as well as the possibility of correcting changes by taking a course of eleutherococcus.

Material and methods. The experiment was conducted on male rats, which were divided into four groups: the first group (n=14) — control, the second group (n=14) — animals treated with eleutherococcus for 30 days, the third group (n=14) — rats, subjected to a single 3-hour “water-immersion dipping”, the fourth group (n=14) — animals that took eleutherococcus for 30 days before the “water-immersion dipping”. The state of the hemostasis system was assessed in all experimental groups. Statistical processing of the results was performed using the Statistica 10.0 package (Statsoft, USA). Significance of differences was assessed using the nonparametric Mann–Whitney test and Student's t-test.

Results. A single immobilization effect was accompanied by hypercoagulable changes, which characterized by shortening of the silicone (p=0.001) and kaolin (p=0.002) time. Completion of the 30-day adaptogen intake followed by emotional immobilization stress was accompanied by an increase in silicone (p=0.001), kaolin (p=0.021), prothrombin (p=0.021) and thrombin (p=0.006) time, which indicated hypocoagulation changes.

Conclusion. The immobilization effect is characterized by hypercoagulability against the background of a decrease in the anticoagulant and fibrinolytic activity of the blood plasma; the course intake of eleutherococcus prior to immobilization is accompanied by hypocoagulant changes and an increase in the anticoagulant activity of the blood plasma.

Keywords: hemostasis system, immobilization, rats, eleutherococcus, emotional stress.

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Background

The modern pace of life inevitably leads to a significant decrease in human motor activity, which can cause neuropsychiatric and cerebral circulation disorders and premature aging [1]. The state of the cardiovascular system is also a contributing factor [2–4].

Prolonged immobilization is linked to negative changes in the hemostasis system [4–7]. Therefore, investigating mechanisms of the maladaptive hemostatic response to emotional immobilization

stress and exploring methods for preventing and correcting its consequences are important. The identification of safe and effective means that can modulate adaptation potential is an urgent direction in contemporary medicine. The use of plant-based adaptogens can enhance performance and increase resistance to various damaging factors [8, 9].

This study aimed to investigate the effect of emotional immobilization stress on the hemostasis system and the potential for correcting these changes through eleutherococcus tincture administration.

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Materials and methods

The study was conducted on 56 male Wistar rats, aged 3 months, divided into four experimental groups of 14 individuals each. Group 1 consisted of control rats. Group 2 received an eleutherococcus tincture for 30 days. Group 3 animals were subjected to a single 3-h water immersion. Group 4 rats were subjected to a single 3-h water immersion after a 30-day pretreatment with eleutherococcus tincture.

Experiments on rats were conducted following Directive 2010/63/EU of the European Parliament (Council of September 22, 2010) on the protection of vertebrate animals used in experiments. Anesthesia and euthanasia of animals were performed in accordance with the Guides to the Care and Use of Experimental Animals. This study was approved by the ethical committee of the Altai State Medical University of the Ministry of Health of Russia (Minute No. 8 dated October 22, 2018).

Rats were subjected to a single acute stressor by immersion in water up to the sternum for 3 h. The rats were placed in small plastic pens made of organic glass, which severely restricted their movement. Water immersion served as an additional emotional stressor for the animals. The water temperature was maintained at a constant +25°C using an ultrathermostat.

An alcoholic extract of eleutherococcus (*Extractum Eleutherococci fluidum*) from a pharmacy was used as an adaptogen. The daily water consumption per 1 kg of rat body weight was measured for 1 week before the adaptogen application. Eleutherococcus tincture was added to individual drinkers based on their daily fluid intake. The adaptogen dose was calculated based on the instructions for use of the drug, which recommend 0.25 mL per 1 kg of body weight per day. To adjust the drug volume and maintain the dosage, the drinking regimen was monitored for 1 month. The course of eleutherococcus intake lasted for 30 days. On the final day of drug administration, the experimental groups were subjected to a single 3-h immobilization period.

Animals were anesthetized with an intraperitoneal injection of telazol solution at a dose of 14 mg/kg of body weight [10]. Blood was then withdrawn from the hepatic sinus into a vacuum system containing sodium citrate solution for the study of hemostasis system parameters.

Blood was centrifuged to obtain platelet-rich and platelet-depleted plasma. Techniques were used to assess the state of the hemostasis system, including the study of platelet aggregation activity, coagulation link of hemostasis, and anticoagulant and fibrinolytic activity.

Diagnostic kits from Technologia-Standard (Russia) were employed as reagents for hemostasis system evaluation using Minilab (Russia) and Trombostat-2 (Germany) coagulometers. Platelet aggregation activity was determined using a Biola aggregometer (Russia).

A working solution of adenosine diphosphate (ADP) in the amount of 0.05 mL was used as an inducer of aggregation using the “ADP™-test” reagent kit.

Antithrombin III activity was evaluated using an SF-46 spectrophotometer from Russia. The index of the contact activation range, which characterizes the activation of the initial phase of blood coagulation, was determined using the formula developed by G.F. Yeregin [11]:

$$\text{CARI (\%)} = \frac{(\text{Silicone time} - \text{kaolin time}) \times 100\%}{\text{Silicone time}},$$

CARI refers to the contact activation range index.

To assess the state of blood fibrinolytic activity [11], spontaneous euglobulin lysis was determined according to Kowarzyk and Buluck using the Fibrinolysis™-test reagent kit. The “SFMC™-test vial” reagent kit was used to determine the level of soluble fibrin–monomer complexes (SFMC). This test can detect latent thrombinemia with increased amounts of SFMC in the blood plasma.

The sensitivity of the thrombin clotting time to heparin was assessed by determining the thrombin–heparin clotting time using the “Heparin™-test” reagent kit. The plasma antithrombin reserve index was calculated according to Bishevsky’s formula [11]:

$$\text{APR (\%)} = \frac{\text{Thrombin–heparin time in the blood plasma of experimental animals} \times 100\%}{\text{Thrombin–heparin time in the plasma of control animals}},$$

APR refers to the antithrombin plasma reserve.

Databases were created using Microsoft Excel 2010 to process and store the experimental data. The statistical software package MedCalc Version 17.9.7 was used for data analysis. The study data are presented as $X \pm m$, where X represents the arithmetic mean and m represents the standard error of the arithmetic mean. Differences were considered reliable at the level of statistical significance $p < 0.05$.

Results

To investigate the effect of *E. eleutherococcus* tincture on the hemostasis system, an experimental group of animals (group 2) that received *E. eleutherococcus* tincture for 30 days but was not subjected to water immersion was created. The silicone

Table 1. Status of the hemostasis system after exposure to a single 3-h water immersion ($X \pm m$)

Hemostasis parameters	Group 1 (n = 14)	Group 2 (n = 14)	Group 3 (n = 14)	Group 4 (n = 14)
Induced platelet aggregation, s	20,2±0,5	25,1±1,2 $p_{1-2}=0,084$	28,4±1,5 $p_{1-3}=0,001$	25,4±3,1 $p_{3-4}=0,211$
Silicone time, s	224,1±7,1	256,2±9,5 $p_{1-2}=0,042$	112,2±10,3 $p_{1-3}=0,001$	190,9±14,0 $p_{3-4}=0,001$
Kaolin time, s	83,9±2,6	119,2±3,9 $p_{1-2}=0,011$	54,3±3,0 $p_{1-3}=0,002$	81,7±4,2 $p_{3-4}=0,021$
Contact activation range index, %	61,2±1,5	50,8±2,9 $p_{1-2}=0,035$	46,4±3,7 $p_{1-3}=0,052$	54,6±3,0 $p_{3-4}=0,064$
Activated partial thromboplastin time, s	22,2±0,5	28,1±0,6 $p_{1-2}=0,034$	17,3±0,9 $p_{1-3}=0,041$	22,5±0,5 $p_{3-4}=0,002$
Prothrombin time, s	13,8±0,3	12,7±0,3 $p_{1-2}=0,078$	10,5±0,4 $p_{1-3}=0,004$	14,1±0,4 $p_{3-4}=0,021$
Thrombin time, s	28,2±0,8	30,1±0,4 $p_{1-2}=0,224$	20,0±0,9 $p_{1-3}=0,005$	44,0±1,7 $p_{3-4}=0,006$
Soluble fibrin–monomer complexes, mg%	3,3±0,1	3,0±0,6 $p_{1-2}=0,251$	10,7±2,5 $p_{1-3}=0,009$	3,0±0,1 $p_{3-4}=0,013$
Fibrinogen, g/L	1,7±0,1	1,4±0,1 $p_{1-2}=0,002$	1,2±0,1 $p_{1-3}=0,012$	1,6±0,2 $p_{3-4}=0,142$

Note: n, number of observations; m, standard error of the arithmetic mean; p, level of statistical significance of the differences in the compared indicators; X, arithmetic mean.

(1.1-fold; $p = 0.042$) and kaolin (1.4-fold; $p = 0.011$) coagulation time and the activated partial thromboplastin time (1.3-fold; $p = 0.034$) increased, indicating hypocoagulation changes. However, the parameters characterizing the external pathway and the final step of coagulation hemostasis did not differ from those of the control group.

After administering eleutherococcus tincture, the level of fibrinogen decreased by 1.2-fold compared with that in the control group ($p = 0.002$). In addition, antithrombin III activity increased 1.2-fold ($p = 0.005$), and the antithrombin reserve in blood plasma remained comparable with the control values. No changes in fibrinolytic activity were observed.

A 3-h water immersion (group 3) resulted in significant changes in the hemostasis system. The experiment showed a 1.4-fold increase in the time of induced platelet aggregation compared with the control group, indicating a decrease in the functional activity of platelets.

Coagulation hemostasis is initiated by the activation of the contact and final stages of coagulation (Table 1). The silicone coagulation time, which reflects the state of the XII coagulation factor at its minimal contact activation, decreased 2-fold after exposure compared with that in the control ($p = 0.001$). In addition, the kaolin coagulation time decreased 1.5-fold ($p = 0.002$), which registers the state of contact factors in conditions of their maximum activation. The exposure described was

also accompanied by a 1.3-fold decrease in the range of the contact activation index ($p = 0.052$).

Hypercoagulative changes were observed, as evidenced by a 1.3-fold decrease in activated partial thromboplastin time compared with the control group ($p = 0.041$) and a 1.4-fold decrease in thrombin time ($p = 0.005$). The SFMC level increased 3.2-fold compared with controls ($p = 0.009$), which may explain the 1.4-fold decrease in fibrinogen ($p = 0.012$) because of its consumption during SFMC formation.

A significant decrease in the heparin cofactor activity of blood plasma ($p = 0.001$) and a 1.2-fold decrease in antithrombin III activity ($p = 0.001$) was found. In addition, fibrinolytic activity decreased, as evidenced by a 1.6-fold increase in the time of spontaneous euglobulin fibrinolysis ($p = 0.007$).

Exposure to emotional immobilization stress resulted in maladaptive changes in both platelet and plasma hemostasis parameters.

The subsequent stage of the study involved evaluating the condition of the hemostasis system in response to water immersion in experimental animals that had previously taken eleutherococcus tincture daily for 30 days, as described earlier (group 4). The control animals were exposed to the same effect as the experimental animals but without the adaptogen.

Despite prior administration of an adaptogen, no changes in vascular thrombotic hemostasis were observed after a single exposure to emotional

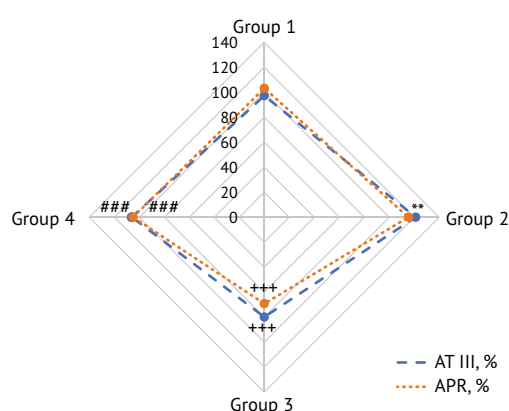


Fig. 1. Changes in rat blood plasma anticoagulant activity after exposure to a single emotional immobilization stressor. AT III, antithrombin III; APR, antithrombin plasma reserve. Group 1, control; group 2, rats taking eleutherococcus tincture for 30 days; group 3, rats after a single emotional immobilization stress; and group 4, rats after a single emotional immobilization stress exposed to eleutherococcus tincture. Statistical significance of the differences: between groups 1 and 2 ** $p < 0.01$; between groups 1 and 3 +++ $p < 0.001$; between groups 3 and 4 ### $p < 0.001$

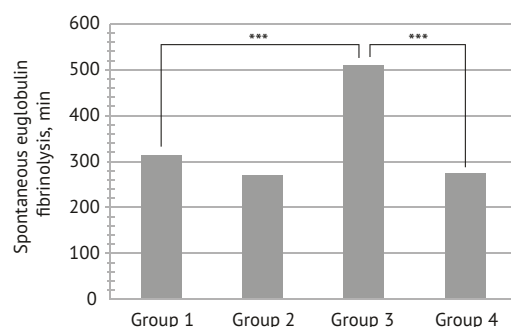


Fig. 2. Changes in fibrinolytic activity of the blood plasma of rats after exposure to a single emotional immobilization stress. Group 1, controls; group 2, rats taking eleutherococcus tincture for 30 days; group 3, rats after exposure to a single emotional immobilization stress; group 4, rats after a single emotional immobilization stress exposed to eleutherococcus tincture; *** $p < 0.001$; *** $p < 0.001$

immobilization stress. However, changes in hypo-coagulation were observed, including a 1.2-fold increase in silicone time ($p = 0.001$), 1.4-fold increase in the kaolin time of blood plasma coagulation ($p = 0.021$), 1.3-fold increase in the activated partial thromboplastin time ($p = 0.002$), and 2.2-fold increase in the prothrombin time ($p = 0.021$) and thrombin time ($p = 0.006$). The amount of SFMC decreased by 3.5-fold compared with the control ($p = 0.013$). This result is comparable to that of intact animals; therefore, the level of this index has been normalized in the experimental animals that were preliminarily given eleutherococcus tincture.

The literature suggests that a decrease in the level of the leading plasma anticoagulant combined with an increased level of SFMC, which is a marker

of thrombinemia, after exposure to acute 3-h water immersion significantly increases the risk of intravascular thrombosis [12].

To prevent thrombus formation, the hemostatic balance must be restored by decreasing SFMC levels and increasing antithrombin III activity. The experiments demonstrate that taking adaptogens before exposure to immobilization stress prevents hypercoagulable shifts, as blood anticoagulant activity remains at control levels (Fig. 1). In the experimental group, the fibrinolytic activity of blood plasma increased 1.9-fold compared with the control level ($p = 0.001$) (Fig. 2).

Therefore, immersion in water for 3 h can lead to hypercoagulable shifts, which may result in intravascular thrombosis. This effect can be mitigated by administering eleutherococcus in advance. Notably, acute emotional immobilization stress did not increase the coagulation potential in group 4.

Discussion

The study revealed that the combination of psychoemotional and immobilization stresses for 3 h resulted in significant hypercoagulable shifts in the plasma hemostasis system both at the initial and final stages of fibrin formation. The SFMC level increased by more than threefold, whereas the fibrinogen content decreased, indicating significant thrombinemia. The natural anticoagulants and fibrinolytic system showed depressed activity, which was not consistent with the coagulation link. A decrease in the functional activity of platelets was also observed, which may be associated with their depletion in the bloodstream of the experimental animals.

Previous studies [12, 13] have demonstrated that thrombinemia in disseminated intravascular coagulation syndrome can lead to thrombocytopenia and consumption thrombocytopenia, which is characterized by platelet hypoaggregation.

This study found that the initial administration of adaptogen prevented the decrease in platelet functional activity caused by exposure to combined stressors. In addition, platelet aggregation function in this group remained at the baseline level. Group 4 had their contact phase of coagulation factors activated to a much lesser extent. Furthermore, most laboratory tests indicated hypo-coagulation changes. The SFMC levels and fibrinogen content did not exceed the physiological values observed in the control group. Signs of adaptogen influence were observed in the natural anticoagulant activity, as evidenced by an increase in the heparin cofactor activity of the blood plasma and antithrombin III. In addition, fibrinolysis was significantly activated in the described group of animals.

Conclusions

1. Exposure to immobilization stress for 3 h resulted in significant changes in hemostatic balance. These changes were characterized by hypercoagulative shifts and the appearance of thrombinemia markers, along with the inhibition of anticoagulant and fibrinolytic activity in the blood plasma.

2. After completing the 30-day course of adaptogen intake, hypocoagulation changes were observed; however, the activity of natural anticoagulants in the blood plasma or fibrinolysis was not inhibited.

3. The administration of eleutherococcus tincture before acute immobilization exposure resulted in a reduction of hypercoagulative shifts, elimination of signs of thrombinemia, and increased anticoagulant activity in the blood plasma.

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