The use of biologics Sedimin® and Gamavit to increase the adaptive capacity of animals

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Abstract. The effect of Gamavit and Sedimin® on the morphological and functional parameters of rats was studied with the short-term introduction of them into the body with sequential exposure to hungry and emotional stress. The dynamics of clinical, physiological and growth indicators of experimental animals is estimated depending on the simulated conditions. Comparative data were obtained on the dependence of the physiological parameters of the organism of experimental animals on the purpose of the studied biogenic preparations.

1. Introduction

The basis of research for the rational use of biological products in the field of keeping productive and decorative animals are experiments to study their interaction and compatibility with basic diets [1]. Due to the different capabilities of animals to adapt to changing environmental conditions, there is not only the need to identify their adaptive capabilities to specific conditions, but also to find ways to reduce the negative effect of stress on the body [2], [3].

The drugs used for stress correction include antioxidants, both natural and synthetic origin, which may differ in the mechanism of action and indications for use [4], [5]. Adaptogens have certain requirements: physiological; the absence of negative side effects with prolonged use; low toxicity. One of the modern drugs that meet all the requirements are Sedimin® and Gamavit.

The purpose of the research is to study the influence of Gamavit and Sedimin® biogenic preparations on the morphophysiological state of rats after their short-term administration into the body under the conditions of a subacute experiment simulating hungry and emotional stress.

Methodology and research methods used in the paper are as follows. A laboratory experiment was carried out using 15 female outbred rats weighing 159.22 ± 6.30 g. Animals were kept under vivarium conditions, the experiment lasted 3 days, during which the animals were kept in free access to water, but in the absence of food, experiencing hungry stress. At the end of the third day, all animals were additionally subjected to emotional stress, in accordance with the “free swimming in a cage” technique. The studied drugs: Sedimin® (Sediminum) - a drug containing iron, iodine, stabilized selenium (manufacturer A-BIO FIRMA LLC, Russia, registration number PVR-2-3.6 / 01651); Gamavit - contains: sodium nucleate, acid denatured emulsified placenta hydrolyzate (PDE), medium 199 (10-fold concentrate) and excipient - water for injection (manufacturer Russia, Micro-Plus CJSC, Russia, registration number PVR-2-3.3 / 01313).

All animals were divided into 3 groups of 5 animals: I - control; II - the animals were injected with the drug Sedimin® once at the beginning of the observations, before being placed under stressful conditions, at a dose of 0.3 ml / 100 g body weight (bw); III - the animals were injected intramuscularly...
with the Gamavit drug once at the beginning of observations, before being placed under stressful conditions, at a dose of 0.3 ml / 100 g body weight (bw). Prior to the observation, blood was taken from all animals by puncture of the tail vein; at the end of the observation, all animals were anesthetized to take blood by direct puncture of the heart. Injections of drugs were carried out in the femoral muscle group. The control group of animals was injected intramuscularly with physiological saline in similar doses and terms. The experiment was carried out in accordance with the principles of good laboratory practice (GOST R 53434-2009) [6].

The studies were carried out using the following methods: hematological - determination of hemoglobin level (Hb, g%) in the blood; leukocyte count (Le, 10⁹ / l) using a Mini-Screen P analyzer (HOSPITEX DIAGNOSTICS, Italy); biochemical - determination of the activity of the enzyme alanine aminotransferase (ALT, U / l) in blood serum; gamma-glutamyltransferase activity (GGT, U / l); total protein content (Pt, g / l); direct bilirubin (BIL-D, µmol / l); the level of iron (Fe, µmol / l) using a biochemical analyzer ARD 200 (Vitako, Russia); mathematical - testing hypotheses about the equality of group means of all quantitative attributes using the nonparametric Wilcoxon-Mann-Whitney criterion. Average values of indicators are given in the form M ± s (M - average, s - standard deviation). Assessment of the statistical significance of differences between the means was carried out at a critical level of p = 0.05.

2. Research results and discussion

2.1. Analysis of blood parameters in the conditions of use of Sedimin®

It was found that the difference in weight loss by the end of the observations in the control group averaged 20.62 ± 2.13 g, and in the group of animals receiving Sedimin® injections - 16.24 ± 1.09 g (p <0.05).

The concentration of hemoglobin in the blood at the time of completion of observations in the group of rats receiving the drug averaged 16.74 ± 0.45 g /%, which is 2.2% more than in intact animals (p> 0.05).

In the control group at the time of completion of the experiment, the leukocyte count was 13.59 ± 0.37 10⁹ / L, which exceeded the similar rats of the second group by 9.1% (p <0.05).

The total protein content in the blood serum of rats receiving injections of the drug increased by 5.9% compared with intact animals (p> 0.05).

Under the influence of stress, the serum activity of GGT and, to a greater extent, ALT in all rats increased, however, in animals that did not receive injection of the studied drug, their activity was higher by 5.1 and 21.6%, respectively (p <0.05). The fact of an increase in ALT and GGT activity during emotional stress was also noted in studies conducted by O. V. Evdokimova and I. V. Gorodetskaya [7]. An increase in GGT activity can lead to negative consequences, since the result of GGT activity is the formation of superoxide anion and hydrogen peroxide, which can stimulate prooxidant reactions [8].

The iron concentration in intact rats was 18.5% higher (p <0.05). When using Sedimin® and Gamavit, an increase in the serum iron content was less pronounced.

It was revealed that the level of direct bilirubin in the blood serum of the control group was 30.2% higher compared to that of the second group (p <0.05). The fact of an increase in the concentration of direct bilirubin in blood serum against the background of an increase in the activity of alanine aminotransferase and gamma-glutamyl transferase suggests that experimental animals suffered damage and destruction of hepatocytes, as well as the development of intrahepatic cholestasis [9]. Moreover, lower GGT and ALT activity in animals treated with Sedimin® injections indicate its positive effect on liver function.

2.2. Analysis of blood parameters in the application of Gamavit

Animals that received an injection of Gamavit, at the time of removal from the experiment exceeded by MT control animals by 3.5% (p> 0.05).
The concentration of hemoglobin in the blood at the time of completion of observations in the group of rats treated with the drug averaged 16.20 ± 0.25 g /%, which is 6.5% less than in intact animals (p <0.05).

In the control group, at the time the experiment was completed, an increase in the leukocyte count to a value of 13.59 ± 0.37 10^9 / L was revealed, which exceeded the similar indicators of rats of the third group by 14.20% (p <0.05).

The total protein content in the blood serum of rats treated with Gamavit injections was 8.19% higher compared with intact animals (p> 0.05).

In animals treated with Gamavit injections, GGT activity was 1.85% lower (p> 0.05) compared with control animals, and ALT activity was 13.92% (p> 0.05).

The iron concentration in rats treated with Gamavit injections was lower by 5.22% (p> 0.05) compared with control.

It was revealed that the level of direct bilirubin in the blood serum of the control group was 27.88% higher compared to that of the third group (p <0.05).

2.3. Comparative evaluation of the use of Sedimin® and Gamavit by blood parameters

It is known that both a decrease and an increase in hemoglobin leads to metabolic disorders in the body, and in extreme cases, to the disease. Various factors can lead to a change in the hemoglobin level, for example: starvation and dehydration, the period of adaptation of the body to new functioning conditions [10-11].

When comparing the effects of Sedimin® and Gamavit, it was found that the hemoglobin level in animals treated with Gamavit was lower by 4.42% compared with rats of the second group (p <0.05). That averaged a difference of 0.74 g /%.

An increase in its content at the end of the subacute experiment in rats of the second and third groups was 6.09 (p <0.05) and 1.75% (p> 0.05), respectively. Moreover, in animals of the control group there was an increase in hemoglobin level by an average of 8.91% (p <0.05).

An increase in hemoglobin level was observed in all animals, which reflects quite logically the simulated experimental conditions (hunger and forced adaptation to new living conditions) that led to an increase in blood viscosity and an increase in hemoglobin level. At the same time, the tested drugs, especially Gamavit, prevent hypochromia.

The content of leukocytes in the blood is a variable indicator and the leukocyte system quickly responds to any changes in the body due to the restructuring of the functioning of the blood-forming organs [12-14].

The leukocyte count at the start of the observations averaged 8.18 ± 1.47 10^9 / L. At the time of completion of the experiment, this indicator in rats of the control group increased on average by 66.11%, and in the second and third groups by 51.00 and 42.52%, respectively (p <0.05).

The established dynamics of hematological parameters in experimental rats testifies to the features of the functioning of the blood system under acute stress. At the same time, the effect of “restraining” the injection of leukocytes into the blood was noted, which prevents the development of leukocytopenia, which was more pronounced in rats that received injections.

The total protein content at the beginning of the experiment averaged 98.45 ± 1.55 g / l. This indicator in all animals due to stressful situations decreased on average from 32.97 to 38.33 g / l (p <0.05). However, the protein level at the end of the experiment was higher in the second group by 5.90% compared with that in the control animals and in the third group by 8.19% (p> 0.05).

A strong decrease in protein levels in experimental animals may indicate a destructive process occurring in the liver under the influence of hunger and emotional stress, the development of which in animals receiving injections of the studied drugs is inhibited.

With some deviations from the normal state, the iron in the blood serum can either decrease or increase. With an increase, a condition called hemochromatosis is observed. In the liver overloaded with iron, the content of free radicals triggering lipid peroxidation (LPO) reactions increases, which leads to damage to both cell membranes and intracellular organelles of liver cells, increased collagen synthesis,
thereby disrupting the structure and function of the organ [15], [16]. There is also evidence that an increased iron content leads to an increased risk of coronary heart disease, and a high level of iron in serum is associated with a risk of death in myocardial infarction [17], [18].

The preparations used also had an effect on the serum iron content during the experiment. So, if at the beginning of the observations its content in the blood serum of rats was 47.42 ± 4.33 μmol / L. Then, after exposure to damaging factors in the control group of rats, its serum content increased on average by 22.00 μmol / L (p <0.05). When using Sedimin® and Gamavit, an increase in the content of iron in blood serum was also noted, however, it was less pronounced. So in the second group, its increase was noted in comparison with the initial indicators by an average of 9.13 μmol / L (p <0.05), and in the third - by 18.38 μmol / L (p <0.05). Thus, we can state the fact that the use of the studied drugs helps to reduce the increase of iron ions in blood serum, which reduces the risk of developing negative effects of the active influence of iron ions on the processes of enhancing lipid peroxidation.

When the liver cells are destroyed, the alanine aminotransferase enzyme enters the bloodstream and its increase is recorded during the analysis.

So, in comparison with the initial indicators, the activity of the ALT enzyme in rats of the control group at the time of completion of observations increased by 36.36%, in animals of the second group by 18.84 and in rats of the third group by 26.07% (p <0.05) However, it should be noted that in animals receiving injections of the studied drugs, the activity of this enzyme was somewhat lower compared to that of the control groups. Moreover, in rats of the second group, against the background of Sedimin® injections, the ALT enzyme activity index was 21.6% lower compared with the control group (p = 0.003). Animals that received Gamavit injections also showed lower levels of activity of this enzyme, however, the difference was statistically unreliable and amounted to 13.9% (p> 0.05).

Bilirubin is a pigment resulting from the breakdown of hemoglobin in the blood. Three main reasons for the increase in bilirubin content can be noted - this is increased destruction of red blood cells, impaired pigment metabolism in the liver, and impaired bile outflow.

It was noted that the content of direct bilirubin in the blood serum before animals were placed in extreme conditions averaged from 2.6 to 3.6 μmol / L. At the end of the experiment, the direct bilirubin BIL-D content was 72.62% higher in the control group compared with the initial data, in the second group - by 60.75 and the third - by 62.03% (p <0.05). The use of Sedimin® had a positive effect on the state of hepatocytes, which was manifested in lower levels of direct bilirubin in the blood serum of rats receiving injections of the drug compared to control animals. So the level of BIL-D in the blood serum of animals of the second group was 30.2% less than that of intact animals (p <0.05).

Gamavit had a similar effect - the level of BIL-D in the blood serum of animals of the third group was lower by 27.88% compared to control animals (p <0.05). There was no significant difference in the effect on the content of this pigment in the blood serum between the tested drugs. Gamma-glutamyltransferase (GGT) is an enzyme involved in the transfer of amino acids across cell membranes in the body. GGT is present in all organs where the processes of absorption and excretion are active (liver, kidneys, pancreas, spleen, etc.). It was noted that an increase in the activity of this enzyme is observed in cases of impaired hepatobiliary system activity.

It was established that before the start of the experiment, the activity of gamma-glutamyltransferase averaged 3.62 ± 0.35 U / L, and by the end of the observations in animals of the control group, this indicator sharply increased to values by 91.63% higher than the initial ones (p <0.05). In the second group, the GGT indicator averaged 41.0 ± 0.71 U / L, which is 91.18% higher compared to the initial data, but 5.1% lower than in control animals (p <0.05). In the third group, at the time of completion of observations, GGT activity averaged 42.40 ± 1.14 U / L, which is 91.47% higher than the baseline, but 1.85% lower than in intact rats (p > 0.05).

3. Conclusion

Intramuscular administration of Sedimin® to laboratory rats under the conditions of a subacute experiment caused significant positive changes in some hematological and biochemical parameters of
blood, and also contributed to maintaining the body weight of animals under conditions of hungry and emotional stress.

The use of Gamavit also had a generally positive effect on the state of stressed animals, while having a less significant effect on maintaining weight-bearing parameters of the animal’s body under simulated conditions.

Thus, we can conclude that the use of test drugs helps to increase the adaptive potential of the body in conditions of damaging factors.

It requires additional research on the effect of various doses of Sedimin® and Gamavit and their possible combined use.

References

[1] Kononenko S I 208 Balancing the diets of pigs using protein feed and biologically active substances (Krasnodar)

[2] Maslova T V and Egorova G G 2008 Problems of ecology and the state of animal health Modern high technology 7 37-8

[3] Haj-Mirzaian A, Amiri S et al 2016 Lithium attenuated the depressant and anxiogenic effect of juvenile social stress through mitigating the negative impact of interleukin-1β and nitric oxide on hypothalamic–pituitary–adrenal axis function Neuroscience 315 271-85

[4] Okunevich I V and Saponov N S 2004 Antioxidants: the effectiveness of natural and synthetic compounds in the complex therapy of cardiovascular diseases Reviews on Clinical Pharmacology and Drug Therapy 3(3) 2-17

[5] Tregubova I A, Kosolapov V A and Spasov A A 2012 Antioxidants: current status and prospects Successes in physiological sciences 43(1) 75-94

[6] Karkishchenko N N and Grachev S V 2010 Manual on laboratory animals and alternative models in biomedical research: a training manual for the system of medical and pharmaceutical postgraduate education (M.: Profile)

[7] Evdokimova O V and Gorodetskaya I V 2013 The influence of experimental hypothyroidism and small doses of L-thyroxine on the activity of aminotransferases and gamma-glutamyltransferase in the blood under the action of stressors of various origin Vestnik of VGMU 12(4) 34-43

[8] Paoliki A A et al 2008 new marker for the development of atherosclerosis and cardiovascular disease - the enzymatic activity of gamma-glutamyl transferase The new Armenian medical journal 2(3) 35-42

[9] Esaulenko E E 2014 Comparative evaluation of the hepatoprotective properties of the pharmaceutical product “Phosphogliv”, linseed oil and oils from the fruits of walnuts and black nuts Modern problems of science and education 2 12311

[10] Liu C-H, Tseng Y-F et al 2018 The changes of red blood cell viscoelasticity and sports anemia in male 24-hr ultra-marathoners Journal of the Chinese Medical Association 81(5) 475-81

[11] Yi S, Hai-Jun W et al 2017 2017 National Trends in Hemoglobin Concentration and Prevalence of Anemia among Chinese School-Aged Children, 1995-2010 The Journal of Pediatrics 183 164-9

[12] Batsigov Kh A et al 2005 Stress Index, Hyperglycemia and Leukocytosis in Acute Mokard Infarction Public Health and Health Care 1-2 153-4

[13] Garmaeva D V and Vasilieva L S 2014 Changes in the myeloid link of the blood system in stressed white rats with experimental hypothyroidism Bulletin of the Buryat State University 4(1) 91-7

[14] Osuka A et al 2019 Natural kinetics of blood cells following major burn: Impact of early decreases in white blood cells and platelets as prognostic markers of mortality Burns 45(8) 1901-7

[15] Allen K J et al 2008 Iron-overload-related disease in HFE hereditary hemochromatosis N. Engl. J. Med. 358(3) 221-30

[16] Washington K M 2009 Liver Modern Surgical Pathology 1 902-59
[17] Gale R P and Butturini A 1994 Stem cells, clonality and leukemia *Hematol. transfusiol.* **39**(6) 3-6

[18] Ghafourian K, Shapiro J S *et al* 2020 Iron and Heart Failure: Diagnosis, Therapies, and Future Directions *JACC: Basic to Translational Science* **5**(3) 300-13