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Toxicological assessment of a bioactive adaptogenic drug for use in rats and pigs

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Glycine and succinic acid elicit a potent stress response. They are not just anti-stress agents; they form a highly effective bioregulatory complex that quickly mobilizes animals' adaptive reserves and significantly reduces the impact of environmental stressors. Their synergistic action achieves a new level of effectiveness when combined with bioactive substances that enhance their physiological effects. The primary focus in this context is the development of a multicomponent adaptogenic drug based on glycine, succinic acid, and a biologically active medium 199, and its toxicological evaluation in rats and pigs. It has been established that a single intragastric administration of the bioactive adaptogenic preparation to male rats at doses of 1000 and 3000 mg/kg body weight did not result in any adverse effects on their clinical condition. Administering a dose of 5000 mg/kg of body weight was accompanied by a transient decrease in motor activity during the first two days after administration. When 9000 and 10000 mg/kg doses were applied to the skin of female rats, mild erythema was observed and disappeared within two hours. Field testing of the therapeutic dose (1.5 mL) demonstrated the drug's high efficacy in rapidly restoring behavioral responses, reducing stress levels, and normalizing physiological parameters in boars after castration. A pathological examination revealed no significant changes in the internal organs except for a probable increase in spleen mass index in female rats that received 2.0 mL on the tenth day, likely due to increased sensitivity to the drug. Rats that received a 1.5 mL dose of the drug demonstrated increased hemoglobin, red blood cell, and total protein levels, as well as lower glucose levels. This is a characteristic manifestation of better adaptation to stressful conditions. Overall, the drug is classified as practically non-toxic in terms of toxicity and as low-toxicity in terms of hazard level. This study will further evaluate adaptogenic anti-stress drugs in mitigating the negative effects of technological stressors on pigs at various stages of the rearing cycle.

Keywords: adaptogenic drug; glycine; succinic acid; biologically active medium 199; internal organ mass index; hematological blood parameters; stress resistance in male piglets.

Introduction

Stress responses can significantly impact the physiological state, behavior, and overall health of animals. Stress in animals can result from various factors, including environmental changes, transportation, lack of social contact, physical exertion, and sound stimuli, such as fireworks or thunderstorms. These factors can lead to behavioral disturbances, loss of appetite, aggression, depression, and other pathological symptoms requiring veterinary care (Martinez-Miro et al., 2016; Liu et al., 2022). Environmental stressors such as heat stress, disrupted feeding schedules, and poor housing conditions can negatively impact health and productivity by impairing the immune system (Bova et al., 2014; Nielsen et al., 2022; Korkh et al., 2023). Short-term acute stress responses can enhance the immune response by increasing T-cell mobilization; however, chronic stress impairs the body's ability to mount an immune response. This includes reducing the immune system's capacity to produce antibodies in response to a vaccine (Dragoș & Tănăsescu, 2010). Heat stress leads to lipid and protein oxidation, reduced water-holding capacity and glycogen stores in muscles, and a shorter shelf life and reduced safety of food products due to increased microbiota (Gonzalez-Rivas et al., 2020).

Studies have demonstrated the positive effect of adding vitamins and trace elements to the diets of pigs and cows in reducing the impact of stressors (Surai et al., 2019; Ali et al., 2020; Smołucha et al., 2024). One of the most effective ways to reduce stress responses in animals is through dietary supplements containing amino acids and organic acids. Glycine, an important amino acid, participates in metabolic processes and directly affects the central nervous system, providing a calming, anti-stress effect (Decker et al., 1995). Transportation typically causes significant stress in goats. Administering ascorbic acid at a dose of 100 mg/kg body weight before transporting goats reduced their excitability. Three hours after transport, the excitability indices of the experimental group did not differ significantly from their pre-transport values. In contrast, the control group of goats, which received only 10 mL/kg of sterile water orally, exhibited significantly lower indices (Ayo et al., 2006). Oregano essential oil reduced serum creatine kinase and cortisol levels in pigs during transport. It also positively affected meat quality and intestinal morphology in pigs after transport stress (Zou et al., 2017). Similar results were obtained when 30 mg/kg of gamma-aminobutyric acid was added to the pigs' diet 74 days before transport (Bi et al., 2014). A necessary component of preclinical studies of new drugs is evaluating their toxicity (Kovalenko et al., 2024). When studying the toxicological characteristics of pharmaceuticals and veterinary drugs, determining acute toxicity is a key step in evaluating their safety for animal health (Matsoso, 2004; Paliy et al., 2024).

The study aimed to conduct a toxicological evaluation of a bioactive adaptogenic drug based on glycine, succinic acid, and biologically active medium 199 in rats and pigs.

Materials and methods

The experiments were conducted in accordance with the current legislation of Ukraine (Law of Ukraine No. 5456-VI of October 16, 2012, "On the Protection of Animals from Cruel Treatment"), as amended as of August 4, 2017, and the "General Ethical Principles for Animal Experiments", adopted by the First National Congress on Bioethics (Kyiv, 2001), and international bioethical standards (materials of the IV European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Stras-

bourg, 1985). An expert opinion from the Commission on Bioethical Review and Ethics of Scientific Research Involving Animals at the Institute of Animal Husbandry of the National Academy of Agrarian Sciences confirmed that the sample collection procedure, feeding conditions, cage housing, administration of inhalation anesthesia, decapitation, and handling of laboratory and experimental animals comply with ethical standards (Protocol No. 15 dated May 20, 2025).

The studies used a bioactive adaptogenic drug that contains the amino acid glycine, succinic acid, and biologically active medium 199 in a single, combined pharmaceutical form. The drug's development and experimental toxicity testing were conducted at the National Scientific Center "Institute of Experimental and Clinical Veterinary Medicine" in Kharkiv.

The scientific rationale for including glycine and succinic acid in the formulation is based on their well-known and proven biological properties. Glycine promotes growth, physiological condition, immune responsiveness, and product quality. It also exhibits a pronounced anti-stress effect. Succinic acid normalizes metabolism, reduces hypoxic symptoms, increases stress resistance, and supports homeostasis. Using it helps increase productivity and accelerate the recovery of animals during stressful periods. Biologically active medium 199 is a standard cell culture medium that contains a balanced complex of inorganic salts, amino acids, vitamins, and glucose, which are necessary to support cell growth and development *in vitro*. In veterinary practice, it is used as an auxiliary component in preparations due to its bioactive properties and ability to enhance the effectiveness of the primary active ingredients.

In vivo studies were conducted on white non-lineage rats to determine the acute toxicity of the experimental drug in accordance with standard procedures (Guideline on Preclinical Safety Studies for Veterinary Medicines). Registration of veterinary medicines: Preclinical safety (www.sahpra.org.za/wp-content/uploads/2020/01/dd5fa0273.05Pre-clinical-safety-Jan04v1.pdf). During the study, the acute toxicity of the test drug samples was determined following oral administration and topical application. The rats were housed under standard vivarium conditions with optimal room microclimate parameters. The air temperature was 20.0 ± 2.0 °C, the relative humidity was 55–65%, and the lighting followed a 14-hour light and 10-hour dark cycle. The air in the room was renewed at least ten times per hour. The housing density was 160 cm^2/animal. The experimental rats were provided with a complete commercial rodent diet and had free access to food and drinking water.

The acute oral toxicity of the experimental drug following a single administration was assessed in 24 male white non-lineage rats aged 3–4 months, with a body weight of 220–250 g. Before the start of the experiment, the rats were kept in quarantine for 15 days, after which three experimental and one control groups, each consisting of 6 rats, were formed based on matching body weight, sex, and age, using a random selection method. Doses of the test drug were calculated individually immediately before administration, based on the rat's body weight, with the volume of the drug administered at one time not exceeding 2.5 cm^3. Administration of the drug to males in the experimental groups was performed based on the absolute mass of the drug in sequential doses of 1000, 3000, and 5000 mg/kg body weight via intragastric administration using an esophagogastric tube. A dose of 5000 mg/kg for such drugs is the threshold for classifying substances as Class IV (low toxicity) and Class V (practically non-toxic). Animals in the control group were administered 2.0 mL of distilled water. The clinical condition of the rats was monitored for 14 days, recording the onset and progression of signs of the drug's toxic effects, the time of death, or the restoration of their physiological condition to normal. During the examination, attention was paid to the rats' behavioral responses, their reactions to external stimuli, the presence or absence of appetite, skin condition, mucosal color, defecation patterns, changes in the color and consistency of feces, and so on. Upon completion of the experiment, the male rats were euthanized. To determine morphological changes in the rodents' internal organs, a postmortem examination was performed, followed by their evaluation using a macroscopic method (Rodrigues et al., 2022).

A study of the acute dermal toxicity parameters of the experimental drug following a single application to the skin was conducted on 42 female white non-lineage rats aged 3–4 months, with a body weight of 200–220 g. For the study, six experimental and one control groups, each consisting of 6 animals, were formed based on matching body weight, sex, and age, using a random selection method. One day before the start of the study, the fur was removed from the intended application site by carefully clipping it with scissors. Subsequently, in the morning before feeding, the preparation was evenly applied to the surface of the previously shaved skin area of the rats (6 × 6 cm) in successive doses of 5000, 6000, 7000, 8000, 9000, and 10000 mg/kg of body weight based on the absolute mass of the drug; animals in the control group received 2.0 mL of distilled water. To prevent the test animals from licking off the solutions, protective collars were placed on them. The observation period lasted 14 days. During this period, the general physiological condition of the females was monitored, the nature of skin lesions at the application site was assessed, and the time of their death or return to a normal state was recorded. The functional condition of the skin at the application site was evaluated based on the intensity of erythema and edema using a scoring scale.

The toxic effects of various doses of the experimental drug on clinical and biochemical blood parameters were assessed following its intramuscular administration to white, non-linear rats. One hundred female rats, aged 3–4 months and weighing 200–220 g, were used in the study. Before the experiment began, the rats were kept in quarantine. After the experiment ended, three experimental groups and one control group were formed based on matching live weight, sex, and age. Each group consisted of 25 rats, which were selected randomly. The rats in the first experimental group were administered an antistress drug solution intramuscularly at a dose of 0.15 ml/kg of body weight (the therapeutic dose). The rats in the second experimental group were administered this solution at 0.75 ml/kg (five times the therapeutic dose), and the rats in the third experimental group were administered 1.5 mL/kg (ten times the therapeutic dose), based on the absolute mass of the drug. The control group females were administered a 0.9% isotonic sodium chloride solution. The therapeutic dose was selected based on generalized data from scientific literature indicating that similar drugs are typically used within a range of 0.10 to 0.30 mL/kg of body weight. It should be emphasized that we deliberately selected an average dose of 0.15 milliliters per kilogram of body weight. The main experimental period lasted 10 days, during which the aftereffects of drug administration were monitored. Daily clinical observations were conducted during the experiment regarding the rats' general condition, appetite, behavioral responses to external stimuli, and appearance. Blood samples were collected from five animals in each group before drug administration and on the fifth, tenth, fifteenth, and twentieth days of the experiment for morphological and biochemical studies and to determine organ mass ratios. The rats were euthanized following deep inhalation anesthesia with chloroform. After euthanasia, a postmortem examination was performed, and macroscopic changes in the internal organs were thoroughly documented. Additionally, the absolute mass of the organs was determined to calculate mass coefficients. Clinical blood tests in rats were performed according to standard methods (Willard & Tvedten, 2012).

The efficacy of administering a therapeutic dose of the experimental drug was checked during the castration of piglets, one of the earliest technological stressors in swine farming, by adjusting the dose to 0.5 mL per kilogram of body weight. This particular stressor was chosen because it could be accurately accounted for individually, whereas other stressors affecting organisms at this age had negligible physiological significance. The piglets were castrated using the open method at 10 days of age. During the procedure, the animals were secured in a "lying" position on a special castration table. The study was conducted on three groups of 10 animals each, matched for genotype, sex, age, and live weight. To identify the selected piglets among the total herd within a single pen, the piglets were marked with a smudge-resistant red zootechnical marker. According to the experimental protocol, the first group of piglets received 0.10 ml/kg of the experimental drug intramuscularly, two days before and two days after castration. The second and third groups received 0.15 and

0.20 mL/kg, respectively. The final dosage was 1.0, 1.5, and 2.0 mL/10 kg of body weight. The experiment lasted 20 days.

The live weight of the piglets was determined by individual weighing on a livestock scale (manufacturer: Guangdong Hostweigh Electronic Technology Co., Ltd., Guangdong, China, 2022), with an accuracy of ± 1–10 g. The live weight and weight of internal organs of the rats were determined by individual weighing on KERN 770-14 laboratory scales (manufacturer: KERN & Sohn GmbH, Balingen, Germany, 2025), with a measurement accuracy of ± 0.2 mg.

In heparin-stabilized rat blood samples, the following were determined: red blood cell count, white blood cell count, white blood cell differential, and hemoglobin concentration; in blood serum – total protein content, glucose level, and the activity of aspartate aminotransferase and alanine aminotransferase enzymes using a Shimadzu UV-1800 spectrophotometer (manufactured by Shimadzu Corporation, Kyoto, Japan, 2020) and standard certified diagnostic reagent kits produced by Reagent PJSC, Dnipro, Ukraine, 2025.

The leukocyte profile of the piglets' blood was assessed by examining Romanovsky-Giemsa – stained smears, followed by a differential count of individual leukocyte types. The total protein and glucose content in the piglets' blood serum was analyzed using a Rayto RT-1904C semi-automatic biochemical analyzer (manufacturer: Rayto Life and Analytical Sciences Co., Ltd., Shenzhen, China, 2021; photometric accuracy ± 1%). Albumin and globulin (alpha-, beta-, and gamma-) fraction content was analyzed using a ULAB 102 spectrophotometer (manufacturer: ULAB Scientific Co., Ltd., Shanghai, China, 2020; photometric accuracy: ± 0.5–1.0%) using certified reagents manufactured by Filisit-Diagnostika LLC (Dnipro, Ukraine, 2025).

Manifestations of stress in young pigs were assessed based on changes in appetite, blood parameters, and visual observations of the condition of the mucous membranes of the oral and nasal cavities, conjunctiva, and skin. Behavioral reactions, as well as live weight and growth rate, were also considered.

The study results were analyzed using Statistica 10.0 software (StatSoft, USA). Results are presented as the mean ± standard deviation (x ± SD). The Tukey test was used to compare differences in mean values between groups, where differences were considered statistically significant at $P < 0.05$ for all data.

Results

In the assessment of acute oral toxicity, a single intragastric administration of the experimental drug at doses of 1000 and 3000 mg/kg body weight was found not to cause any abnormalities in the physiological condition or behavior of the rats compared to the control group. The rats maintained normal mobility, responded appropriately to external stimuli, and actively consumed food and water. The color of their visible mucous membranes was normal, and their fur was smooth and shiny. No digestive disorders were observed, and the feces were normal in color and consistency.

In contrast, administration of the drug at a dose of 5000 mg/kg body weight was accompanied by mild suppression of motor activity in the rats during the first two days of the experiment, compared to the control group, which subsequently recovered completely. Their fur appeared dull and ruffled, while the condition of the skin and mucous membranes remained normal. No changes in the color or consistency of feces were observed, and food and water intake remained at the level of the control group. Given that the rats receiving the drug at a dose of 5000 mg/kg body weight exhibited the most noticeable changes in symptoms, a decision was made to euthanize them, followed by an assessment of morphological changes in internal organs and the identification of manifestations of acute drug toxicity. During the external examination of the euthanized rats, it was found that their fur retained its natural color and was free of contamination, and the visible mucous membranes were pink in color.

Postmortem examination of the rats revealed no significant changes to their internal organs. The heart was cone-shaped with no signs of enlargement and contained blood clots. The lungs were pink and had a normal shape. The liver was dark cherry-colored and firm. The spleen and pancreas appeared normal. The kidneys were light brown, not enlarged, and had a distinct pattern when viewed cross-sectionally. The small and large intestines showed no morphological abnormalities, though the stomach contained a small amount of food.

Since no cases of intoxication or death were recorded in the control or experimental groups during the experiment, the LD_{50} of the anti-stress drug could not be calculated. However, it is likely to exceed 5000 mg/kg of body weight. In accordance with the requirements of SOU 85.2-37-736:2011, the anti-stress preparation can be classified as Class V – practically non-toxic substances (LD_{50Oral} 5001–15000 mg/kg body weight) – and, according to the degree of hazard, as Class IV – low-toxicity substances (LD_{50Oral} >5000 mg/kg body weight).

Based on the results of the acute dermal toxicity assessment of the anti-stress drug after a single application to the skin, there were no significant differences in the clinical condition, behavior, or appearance of the rats in the experimental groups compared to the control group. The female rats responded appropriately to noise and light, and their skin showed no signs of pain, wrinkling, swelling, thickening, or formation of scales. Moderate skin erythema was observed only in animals treated with doses of 9000 and 10000 mg/kg body weight, rated as a one-point reaction on the scale, which fully resolved within two hours after application. Since all females survived until the end of the experiment, the LD_{50} could not be calculated for topical application of the anti-stress drug at doses of 5000–10000 mg/kg body weight. The LD_{50} likely exceeds 10000 mg/kg body weight. In accordance with the requirements of SOU 85.2-37-736:2011, the anti-stress agent can be classified as Class V based on its skin irritation potential. This classification is for practically non-toxic substances (LD_{50Cut} 2821–22600 mg/kg). According to the degree of hazard, the agent is classified as Class IV, which is for low-toxicity substances (LD_{50Cut} > 2500 mg/kg).

The results of toxicological studies indicate that intramuscular administration of various doses of the anti-stress drug to female rats over a twenty-day observation period did not result in any fatalities. All rats, regardless of group, remained active, exhibited a good appetite, and consumed water normally.

The coat remained dense, smooth, shiny, and lay evenly against the body surface. Behavioral responses to external sound and light stimuli corresponded to the natural species norm; there were no signs of diarrhea.

During the postmortem examination, no significant deviations in the size, condition, or color of the mucous membranes of the esophagus, stomach, and small and large intestines were observed in the female experimental groups compared to the control group. However, analysis of the determined internal organ mass ratios indicates minor intergroup differences (Table 1). In the third experimental group of females, a statistically significant increase in spleen mass index was observed on the tenth day after drug administration: 60.7% ($P < 0.001$) compared to the control group, and 40.6% ($P < 0.05$) and 32.4% compared to the first and second experimental groups, respectively. During this period, a significant increase in the spleen mass index was also observed compared to the values on day 5 of the experiment: 41.6% in group II and 60.7% in group III ($P < 0.001$). Thus, the spleen proved to be the organ most sensitive to the potential toxic effects of the drug in female rats. After discontinuing administration, the mass ratios of all internal organs in the female experimental groups mostly approached the values of the control group or remained slightly elevated. This indicates an adaptive response and the absence of significant toxicological effects from the anti-stress drug.

Notably, compared to the control group, the levels of red blood cells and hemoglobin in the blood of female rats in the experimental groups did not significantly change before or after drug administration (Table 2). In contrast, the results of the studies revealed a significant decrease in white blood cell count in the blood of rats in Group III on the fifth and tenth days of the experiment: 20.3% ($P < 0.01$) and 14.3%, respectively, compared to the control group. For females in groups I and II, this indicator was significantly lower by 22.0% ($P < 0.05$) and 15.1% ($P < 0.001$) on the fifth day, and by 21.0% ($P < 0.01$) and 8.2% on the tenth day, respectively. At the same time, the data

analysis indicates a clear trend toward an increase in hemoglobin concentration of 1.0–5.0% and 11.2–19.3% in the rats of the experimental groups on the fifth and tenth days, respectively, compared to their age-matched counterparts in the control group. However, by the fifteenth day of observation, these values did not differ significantly among females. The red blood cell count remained relatively unchanged throughout the experiment in all groups, with a slight tendency toward an increase in females of Group I starting from the tenth day.

Table 1

Changes in the mass ratios of internal organs in female rats following 10 days of intramuscular administration of an anti-stress drug (x ± SD, n = 5)

Study period	Group/dose of the drug, mL	Internal organ mass ratio, % of body weight				
		heart	spleen	liver	lungs	kidneys
5 days after administration of the drug	control	0.39 ± 0.02[a]	0.28 ± 0.01[a]	4.08 ± 0.18[a]	1.04 ± 0.10[a]	0.65 ± 0.02[a]
	I – 0.15	0.36 ± 0.02[a]	0.24 ± 0.04[a]	3.83 ± 0.11[a]	0.87 ± 0.08[a]	0.63 ± 0.01[a]
	II – 0.75	0.34 ± 0.03[a]	0.24 ± 0.04[a]	3.71 ± 0.14[a]	0.83 ± 0.05[a]	0.51 ± 0.09[a]
	III – 1.50	0.40 ± 0.06[a]	0.29 ± 0.02[a]	4.17 ± 0.15[a]	1.05 ± 0.11[a]	0.65 ± 0.02[a]
10 days after administration of the drug	control	0.41 ± 0.04[a]	0.28 ± 0.02[a]	4.03 ± 0.10[a]	0.88 ± 0.08[a]	0.60 ± 0.03[a]
	I – 0.15	0.37 ± 0.01[a]	0.32 ± 0.04[a]	4.11 ± 0.14[a]	0.97 ± 0.09[a]	0.66 ± 0.05[a]
	II – 0.75	0.34 ± 0.02[a]	0.34 ± 0.05[a]	3.96 ± 0.22[a]	0.86 ± 0.02[a]	0.57 ± 0.01[a]
	III – 1.50	0.34 ± 0.03[a]	0.45 ± 0.02[b]	4.35 ± 0.26[a]	0.88 ± 0.07[a]	0.67 ± 0.05[a]
5 days after discontinuation of the drug	control	0.36 ± 0.03[a]	0.27 ± 0.04[a]	3.91 ± 0.19[a]	0.89 ± 0.07[a]	0.65 ± 0.02[a]
	I – 0.15	0.40 ± 0.03[a]	0.30 ± 0.06[a]	3.85 ± 0.11[a]	0.95 ± 0.10[a]	0.69 ± 0.06[a]
	II – 0.75	0.34 ± 0.01[a]	0.26 ± 0.04[a]	3.77 ± 0.09[a]	0.86 ± 0.02[a]	0.62 ± 0.04[a]
	III – 1.50	0.42 ± 0.02[a]	0.30 ± 0.01[a]	4.02 ± 0.08[a]	1.04 ± 0.09[a]	0.68 ± 0.02[a]
10 days after discontinuation of the drug	control	0.33 ± 0.05[a]	0.28 ± 0.03[a]	3.94 ± 0.21[a]	0.92 ± 0.09[a]	0.66 ± 0.02[a]
	I – 0.15	0.35 ± 0.02[a]	0.26 ± 0.01[a]	3.86 ± 0.12[a]	1.02 ± 0.08[a]	0.61 ± 0.03[a]
	II – 0.75	0.33 ± 0.02[a]	0.26 ± 0.03[a]	3.73 ± 0.12[a]	0.91 ± 0.07[a]	0.61 ± 0.01[a]
	III – 1.50	0.38 ± 0.01[a]	0.28 ± 0.02[a]	4.11 ± 0.07[a]	1.02 ± 0.05[a]	0.69 ± 0.03[a]

Note: different letters indicate values that differ significantly from one another within the same column.

Table 2

Changes in key hematological parameters in rats following 10 days of intramuscular administration of an anti-stress drug (x ± SD, n = 5)

Indicator	Group/dose of the drug, mL	Study period, day				
		before administration	5	10	15	20
Hemoglobin concentration, g/dm^3	control	137.6 ± 4.4[a]	135.0 ± 2.7[a]	132.3 ± 10.8[a]	129.6 ± 5.4[a]	143.1 ± 8.1[a]
	I – 0.15	139.6 ± 4.2[a]	141.7 ± 8.1[a]	157.9 ± 9.4[a]	137.6 ± 2.7[a]	153.9 ± 5.4[a]
	II – 0.75	137.8 ± 2.2[a]	137.6 ± 4.1[a]	153.8 ± 9.4[a]	134.6 ± 2.8[a]	149.8 ± 2.7[a]
	III – 1.50	136.9 ± 6.3[a]	136.3 ± 4.0[a]	147.1 ± 4.1[a]	128.2 ± 4.0[a]	148.5 ± 8.1[a]
Red blood cell count, 10^{12}/dm^3	control	5.90 ± 0.02[a]	5.90 ± 0.01[a]	6.12 ± 0.12[a]	6.52 ± 0.02[a]	5.63 ± 0.15[a]
	I – 0.15	5.96 ± 0.01[a]	6.05 ± 0.02[a]	6.44 ± 0.12[a]	6.78 ± 0.08[a]	5.87 ± 0.12[a]
	II – 0.75	6.02 ± 0.02[a]	6.05 ± 0.06[a]	6.24 ± 0.15[a]	6.71 ± 0.05[a]	5.65 ± 0.11[a]
	III – 1.50	5.88 ± 0.04[a]	6.02 ± 0.06[a]	6.17 ± 0.07[a]	6.61 ± 0.19[a]	5.61 ± 0.13[a]
White blood cell count, 10^9/dm^3	control	9.22 ± 0.36[a]	9.41 ± 0.58[b]	8.75 ± 0.58[b]	7.89 ± 0.11[a]	7.83 ± 0.33[a]
	I – 0.15	9.44 ± 0.28[a]	9.62 ± 0.79[b]	8.83 ± 0.25[b]	8.23 ± 0.41[a]	8.04 ± 0.29[a]
	II – 0.75	9.52 ± 0.12[a]	9.49 ± 0.66[b]	8.17 ± 0.33[b]	7.50 ± 0.16[a]	7.92 ± 0.50[a]
	III – 1.50	9.12 ± 0.34[a]	7.50 ± 0.16[a]	7.50 ± 0.16[a]	7.50 ± 0.16[a]	7.86 ± 0.21[a]

Note: see Table 1.

The study revealed changes in hemoglobin levels at the end of the experiment compared with baseline values. There were increases of 11.8% and 11.3% in Groups I and II, respectively ($P < 0.05$), relative to Day 15. Meanwhile, the concentration of hemoglobin in Group III was 15.8% higher ($P < 0.05$). Regarding red blood cells, a statistically significant increase in concentration was observed on Day 10 in Group I rats by 6.4%, compared to Day 5, and on Day 15 in Group II rats by 7.5%, compared to Day 10. On day 20, a decrease in erythrocyte count was observed in all groups, including the control group, by 13.6%, 13.4%, 15.7%, and 15.1%, respectively ($P < 0.05$), compared to day 15. The drug had the most pronounced effect on white blood cell count when the highest dose was administered. As early as the fifth day of the experiment, a 17.8% decrease in this parameter was recorded ($P < 0.001$), compared to the value before administration.

When comparing the biological effects of different doses of the adaptogenic drug, statistically significant differences were observed on the 10th day of the experiment regarding white blood cell count, with females in Group III showing a 15.1% reduction ($P < 0.001$) compared to the corresponding value in Group I.

The leukocyte formula in the blood of rats in the experimental groups did not change significantly throughout the experimental period compared with the control group. It was established that, before drug administration, the leukocyte formula in the blood of rats across all groups was nearly identical, indicating a balanced physiological state before the start of the experiment. On the fifth day after drug administration, a slight increase in the proportion of eosinophils and a decrease in lymphocytes were observed in the blood of all experimental groups, while the level of neutrophils remained stable (Table 3). By the tenth day of the experiment, the leukocyte profile in the blood of the rats in the experimental groups had normalized, both relative to their age-matched counterparts in the control group and compared to the previous observation period. However, a slight increase in lymphocyte count and normalization of neutrophil levels were observed, indicating the development of adaptive processes following the initial reaction to drug administration. On the fifteenth and twentieth days, the differences between groups were minimal, and white blood cell counts remained within physiological norms, indicating an absence of significant toxic effects on the rats' immune systems.

Table 4 shows that, throughout the entire experiment, the concentration of total protein in the blood serum of females in the first experimental group significantly exceeded the values in the control and third experimental groups: by 11.9% ($P < 0.05$) and 12.8% ($P < 0.001$) on the fifth day, by 5.6% ($P < 0.05$) and 5.8% ($P < 0.05$) on the tenth day, by 6.5% ($P < 0.05$) and 6.5% ($P < 0.05$) on the fifteenth day, and by 5.9% ($P < 0.05$) and 5.9% ($P < 0.05$) on the twentieth day. Additionally, on the fifth day, a significant increase in this indicator was observed in the first experimental group compared to the previous study results, at 12.6%. Elevated glucose levels were recorded only in the control group.

On the fifth day of the experiment, a statistically significant increase in the activity of the liver enzyme aspartate aminotransferase was observed in the blood serum of females in the control and third experimental groups, compared with rats in the first and second experimental groups, by 8.6% ($P < 0.001$) and 13.8% ($P < 0.001$) and

11.1% (P < 0.001) and 16.4% (P < 0.001), respectively. A comparison of this indicator's level with values from the previous study period also revealed a 27.6% increase (P < 0.001) in rats of group III on the fifth day after drug administration, against a 22.1% increase (P < 0.001) in enzyme activity in animals of the control group.

The third experimental group of females had the highest alanine aminotransferase levels, likely due to the increased dose of the experimental drug. On the fifth day of the experiment, their levels were 29.2% higher than those of experimental group I (P < 0.001) and 33.8% higher than those of experimental group II (P < 0.001). The differences between the control group and the latter two groups were 21.2% (P < 0.001) and 25.4% (P < 0.001), respectively. By the tenth day, the differences decreased: between groups III and I and II, to 12.9% and 16.9%, respectively, and between the control group and groups I and II, to 9.7% and 13.6%, respectively. However, these differences only reached statistical significance at the P < 0.01 level in comparisons with the control group. The most significant changes in ALT activity, compared with the previous study's results, were observed on the fifth day of the experiment. On that day, ALT activity increased by 16.8% (P < 0.001), 8.5% (P < 0.05), and 15.4% (P < 0.05), respectively, in the control group and the first and third experimental groups.

Table 3

Changes in the white blood cell count in rats following 10 days of intramuscular administration of an anti-stress drug (x ± SD, n = 5)

Study period	Group/dose of the drug, mL	Leukocyte formula, %					
		rod-shaped neutrophils	segmented neutrophils	eosinophils	basophils	monocytes	lymphocytes
Before administration of the drug	Control	1.00 ± 0.00	29.00 ± 0.66	3.66 ± 0.66	0.0 ± 0.0	3.00 ± 0.66	63.34 ± 0.33
	I – 0.15	1.33 ± 0.33	28.33 ± 1.00	3.66 ± 1.00	0.0 ± 0.0	3.33 ± 0.66	63.35 ± 0.33
	II – 0.75	1.00 ± 0.00	29.66 ± 0.33	4.00 ± 0.66	0.0 ± 0.0	3.00 ± 0.66	62.34 ± 1.33
	III – 1.50	1.33 ± 0.33	28.66 ± 1.00	3.66 ± 0.33	0.0 ± 0.0	2.66 ± 0.66	63.69 ± 1.33
5 days after administration of the drug	Control	2.00 ± 0.66	25.66 ± 1.00	7.66 ± 0.33	00.0 ± 0.0	4.00 ± 0.66	60.68 ± 1.00
	I – 0.15	1.66 ± 0.33	26.66 ± 0.33	7.33 ± 0.33	1.00 ± 0.00	4.33 ± 0.33	59.02 ± 0.66
	II – 0.75	1.66 ± 0.66	25.66 ± 1.33	7.33 ± 1.00	0.66 ± 0.00	3.33 ± 0.33	61.36 ± 2.33
	III – 1.50	2.00 ± 0.00	26.00 ± 0.66	6.66 ± 0.00	0.0 ± 0.0	3.66 ± 0.66	61.68 ± 0.66
10 days after administration of the drug	Control	2.33 ± 0.33	27.00 ± 0.66	7.33 ± 0.33	0.66 ± 0.00	4.66 ± 0.33	58.02 ± 0.66
	I – 0.15	1.33 ± 0.33	26.33 ± 1.00	6.33 ± 1.00	0.0 ± 0.0	4.66 ± 1.00	61.35 ± 1.00
	II – 0.75	1.66 ± 0.33	27.00 ± 1.33	7.33 ± 0.33	0.33 ± 0.00	4.33 ± 1.00	59.35 ± 1.66
	III – 1.50	1.66 ± 0.33	26.66 ± 1.00	6.66 ± 0.66	0.0 ± 0.0	4.33 ± 1.33	60.69 ± 1.33
15 days after administration of the drug	Control	1.00 ± 0.33	27.00 ± 1.33	6.33 ± 0.66	0.66 ± 0.00	3.66 ± 0.33	61.35 ± 2.00
	I – 0.15	1.66 ± 0.33	26.33 ± 1.00	7.33 ± 0.66	0.00 ± 0.00	4.66 ± 1.00	60.02 ± 1.66
	II – 0.75	1.33 ± 0.33	26.33 ± 1.00	5.66 ± 1.00	1.00 ± 0.00	4.00 ± 0.66	61.68 ± 1.00
	III – 1.50	1.00 ± 0.00	26.66 ± 1.00	6.00 ± 1.33	0.0 ± 0.0	4.33 ± 1.00	62.01 ± 0.66
20 days after administration of the drug	Control	1.66 ± 0.33	27.00 ± 1.00	5.33 ± 0.33	1.00 ± 0.00	3.00 ± 0.00	62.01 ± 0.66
	I – 0.15	1.33 ± 0.33	27.33 ± 1.00	5.33 ± 1.00	0.0 ± 0.0	4.00 ± 0.66	62.01 ± 2.00
	II – 0.75	1.66 ± 0.33	26.33 ± 1.00	6.00 ± 0.66	0.0 ± 0.0	4.33 ± 0.33	61.68 ± 1.66
	III – 1.50	2.00 ± 0.00	26.66 ± 1.33	5.66 ± 1.00	0.33 ± 0.33	3.66 ± 0.66	61.69 ± 0.66

Note: none of the samples differ from one another within a column.

Table 4

Changes in the levels of biochemical markers in rat serum following 10 days of intramuscular administration of an anti-stress drug (x ± SD, n = 5)

Indicator	Group/dose of the drug, mL	Before administration	Study period, day			
			5	10	15	20
Total protein content, g/dm³	control	64.48 ± 1.26^{a}	61.88 ± 2.86^{a}	63.54 ± 0.71^{a}	65.69 ± 1.42^{a}	63.54 ± 0.71^{a}
	I – 0.15	61.46 ± 0.84^{a}	69.23 ± 0.69^{b}	67.11 ± 1.42^{b}	69.96 ± 0.71^{b}	67.30 ± 1.21^{b}
	II – 0.75	62.34 ± 2.12^{a}	64.26 ± 1.43^{a}	65.68 ± 1.42^{a}	67.11 ± 1.42^{a}	65.33 ± 2.14^{a}
	III – 1.50	64.22 ± 1.24^{a}	61.40 ± 0.72^{a}	62.83 ± 1.43^{a}	65.68 ± 1.42^{a}	63.55 ± 0.71^{a}
Glucose level, mmol/dm³	control	6.06 ± 0.02^{a}	6.12 ± 0.34^{a}	5.52 ± 0.43^{a}	5.61 ± 0.17^{a}	5.78 ± 0.35^{a}
	I – 0.15	6.12 ± 0.08^{a}	5.87 ± 0.26^{a}	5.25 ± 0.35^{a}	5.43 ± 0.35^{a}	5.61 ± 0.09^{a}
	II – 0.75	6.02 ± 0.06^{a}	5.87 ± 0.09^{a}	5.22 ± 0.26^{a}	5.34 ± 0.26^{a}	5.52 ± 0.43^{a}
	III – 1.50	6.10 ± 0.02^{a}	6.04 ± 0.17^{a}	5.52 ± 0.17^{a}	5.60 ± 0.26^{a}	5.61 ± 0.35^{a}
ALT activity, μmol/h·cm³	control	2.26 ± 0.02^{a}	2.64 ± 0.06^{ab}	2.55 ± 0.09^{a}	2.52 ± 0.12^{a}	2.58 ± 0.06^{a}
	I – 0.15	2.24 ± 0.02^{a}	2.43 ± 0.05^{a}	2.49 ± 0.08^{a}	2.49 ± 0.03^{a}	2.55 ± 0.02^{a}
	II – 0.75	2.28 ± 0.06^{a}	2.32 ± 0.08^{a}	2.35 ± 0.06^{a}	2.49 ± 0.03^{a}	2.49 ± 0.03^{a}
	III – 1.50	2.34 ± 0.08^{a}	2.70 ± 0.09^{b}	2.59 ± 0.07^{b}	2.51 ± 0.06^{a}	2.54 ± 0.06^{a}
AST activity, μmol/h·cm³	control	4.08 ± 0.12^{a}	4.98 ± 0.20^{ab}	4.51 ± 0.11^{ab}	3.63 ± 0.13^{a}	3.66 ± 0.20^{a}
	I – 0.15	4.20 ± 0.08^{a}	4.11 ± 0.06^{a}	4.11 ± 0.06^{a}	3.56 ± 0.27^{a}	3.87 ± 0.07^{a}
	II – 0.75	4.12 ± 0.06^{a}	3.97 ± 0.07^{a}	3.97 ± 0.17^{a}	3.59 ± 0.06^{a}	3.63 ± 0.06^{a}
	III – 1.50	4.16 ± 0.02^{a}	5.31 ± 0.26^{b}	4.64 ± 0.30^{b}	3.66 ± 0.09^{a}	3.73 ± 0.13^{a}

Note: different letters indicate values that differ significantly from one another within the same column.

AST activity decreased more rapidly than ALT activity, as evidenced by their ratio narrowing to 1.4–1.5 versus 1.8–1.9 by the 20th day of the experiment.

The recorded changes in serum biochemical parameters indicate the drug had a negligible toxic effect on the rats, which remained within normal limits and gradually normalized ten days after discontinuing administration.

Visual observations of the piglets before castration revealed noticeable behavioral differences among some litters. However, the majority of the young stock exhibited motor activity that remained within the physiological range of daily fluctuations, showing no significant differences among them. The piglets were active, periodically changing their location within the pen. They interacted calmly with their mothers and without significant conflict with their peers while nursing. After feeding, they settled down to rest, huddling closely together. Their skin had a natural sheen and was free of damage. The conjunctiva of their eyes was smooth, their mucous membranes were clean and pink, and their breathing was even, indicating an absence of significant stress. The piglets remained relatively calm during drug administration. Blood parameters within the groups formed prior to castration showed no significant differences and were within physiological norms (Table 5).

Table 5
Changes in hematological parameters in animals exposed to various doses of the experimental drug following castration (x ± SD, n = 10)

Indicator	Period of drug administration	Group		
		I	II	III
Dose administered before castration, mL		0.1	0.15	0.20
Dose administered at the end of the experiment, mL		1.0	1.5	2.0
Erythrocytes, 10^{12}/L	before castration	5.22 ± 0.17[a]	5.31 ± 0.62[a]	5.36 ± 0.37[a]
	at the end of the experiment	5.58 ± 0.64[a]	5.63 ± 0.81[a]	5.46 ± 0.73[a]
Leukocytes, 10^{9}/L	before castration	12.63 ± 0.85[a]	12.73 ± 0.84[a]	12.92 ± 0.79[a]
	at the end of the experiment	12.74 ± 0.56[a]	12.82 ± 0.93[a]	12.86 ± 0.59[a]
Hemoglobin, g/L	before castration	116.3 ± 5.3[a]	115.9 ± 2.4[a]	115.5 ± 3.5[a]
	at the end of the experiment	118.5 ± 4.2[a]	118.9 ± 4.8[a]	117.6 ± 3.7[a]
Total protein, g/L	before castration	62.41 ± 0.52[a]	62.28 ± 0.66[a]	62.25 ± 0.73[a]
	at the end of the experiment	64.25 ± 0.52[a]	65.38 ± 0.49[a]	64.12 ± 0.50[a]
Albumin, %	before castration	47.3 ± 1.7[a]	46.9 ± 1.6[a]	47.9 ± 2.9[a]
	at the end of the experiment	46.7 ± 1.4[a]	46.4 ± 1.7[a]	46.9 ± 2.5[a]
Globulins, %	before castration	52.7 ± 2.6[a]	53.1 ± 2.4[a]	52.1 ± 1.8[a]
	at the end of the experiment	53.3 ± 1.8[a]	53.6 ± 2.0[a]	53.0 ± 1.6[a]
Glucose, mmol/L	before castration	3.46 ± 0.07[a]	3.48 ± 0.09[a]	3.51 ± 0.05[a]
	at the end of the experiment	3.87 ± 0.05[b]	3.64 ± 0.07[a]	3.89 ± 0.05[b]

Note: different letters denote values that differ significantly from one another within a range of six rows.

Immediately after castration, noticeable behavioral changes were observed in all groups of piglets. They became more nervous, avoided contact with their peers, and lay down for long periods. Additionally, a temporary decrease in appetite was observed. However, the animals' response to castration varied depending on the drug dose; the highest dose did not produce the greatest effect as expected.

No significant changes were observed in the hematological profile of the piglets' blood at the end of the experiment compared to the data obtained prior to castration. However, a dose of 1.5 mL per 10 kg of body weight was found to be the most effective during this period. After receiving this dose, the piglets were able to tolerate the stressor more easily and returned to normal motor activity and appetite more quickly. The piglets exhibited the lowest level of anxiety, a shorter resting time, and a slight increase in red blood cell count (0.9–3.1%), hemoglobin concentration (0.4–1.1%), and total blood protein content (1.8–2.0%). Meanwhile, their glucose levels decreased by 5.9–6.4%. All of these parameters remained within the physiological norm. At the same time, the difference in serum glucose levels between the piglets in Group II and those in Groups I and III was statistically significant ($P < 0.01–0.05$), indicating the former's better adaptive capacity to castration-induced stress.

These results are supported by data on live weight and weight gain in the experimental animals (Table 6). In particular, based on the results of individual weighing of the boars, it was found that at the beginning of the experiment, no significant difference in live weight was observed between the groups, whereas by the end, it had increased slightly by 0.8% and 2.5% in animals of Group II, accompanied by an increase in their average daily gain of 2.3% and 7.1% ($P < 0.05$), compared to their peers in Groups I and III.

Table 6
Dependence of live weight and growth rate of young boars on the dose of the experimental drug (x ± SD, n = 10)

Indicator	Group		
	group I	group II	group III
Dose administered before castration, mL	0.10	0.15	0.20
Dose administered at the end of the experiment, mL	1.0	1.5	2.0
Live weight at the start of the experiment, kg	2.98 ± 0.49[a]	2.94 ± 0.53[a]	3.06 ± 0.27[a]
Live weight at the end of the experiment, kg	7.42 ± 0.21[a]	7.48 ± 0.36[a]	7.30 ± 0.42[a]
Absolute weight gain, kg	4.44 ± 0.68[a]	4.54 ± 0.24[a]	4.24 ± 0.75[a]
Average daily weight gain, g	222.0 ± 4.5[a]	227.0 ± 2.9[b]	212.0 ± 4.7[a]

Note: different letters denote values that differ significantly within a single row of the table.

Discussion

One of the challenges facing modern animal husbandry is the mismatch between certain technological and environmental conditions and the biological needs of animals required to achieve high production performance, which leads to the development of stress responses (Geetanjali et al., 2023; Korkh et al., 2025). The primary method of preventing stress and its consequences today is pharmacological intervention. To address stress, which in turn leads to behavioral disorders, various medications are used, including psychotropic, anticonvulsant, and antihistamine drugs, hormones, analgesics, and neuroleptics (Poroshinska et al., 2024). However, all of these affect the body's immune function (Puangsri et al., 2023).

Under the conditions of our experiment, the drug was systematically absorbed and distributed throughout the tissues. An analysis of the morphofunctional state of the organs revealed that the spleen, being the central organ of the immune system, was more sensitive to toxic effects, as shown by an increase in its mass index. *In vivo* studies using laboratory animals administered Mahā Pigut Triphala (Intatham et al., 2024) have shown significant differences in the absolute mass of certain internal organs, particularly the heart, spleen, and kidneys. However, these variations in organ mass fell within the normal reference value range. Thus, they may be the result of the animals' individual characteristics rather than the influence of the substances under study. In contrast, other researchers (Chen et al., 2018) have demonstrated that no significant deviations in physiological parameters or pathological changes in the major organs of rats were observed during chronic or acute toxicity testing of the drugs.

Wysokiński & Szczepocka (2018) report a link between stress development and red blood cell parameters. An increase in red blood cell count was observed in stressed animals. However, based on a summary of our studies, no such change in red blood cell count was detected. Since red blood cell count is related to hemoglobin levels, we studied the dynamics of changes in the latter. According to our data, there was a clear trend toward an increase in hemoglobin concentration of 1.0–5.0% and 11.2–19.3% in rats in the experimental groups on the fifth and tenth days of the experiment compared to age-matched rats in the control group. However, the decrease in hemoglobin levels in the blood of the control group rats may indicate the development of hemodilution, a relative decrease in hemoglobin concentration noted in the work of Engel et al. (2024), or it may be considered a compensatory reaction rather than a pathological shift.

Since different classes of anti-stress drugs can exhibit both pro- and anti-inflammatory effects, we examined white blood cell counts

(Lynall et al., 2020). In addition, both the development of stress itself and treatment with relevant drugs are associated with changes in white blood cell counts. Beydoun et al. (2024) have shown a decrease in blood leukocyte counts, which was also observed in our studies during blood analysis of rats in Group III and may indicate a possible redistribution of cells in the blood or an effect on immune regulation. Based on this, the ratio of different types of leukocytes (the leukocyte differential count) was examined. In research conducted by Suresh & Koner, 2012; Sealock et al. (2023), the development of stress was inversely correlated with the number of neutrophils, lymphocytes, and eosinophils, highlighting an immunosuppressive profile linked to cortisol and cytokine levels.

Obeagu (2025) found that the body's stress response involves a decrease in the number of neutrophils, monocytes, and basophils, as well as the activation of the hypothalamic-pituitary-adrenal axis and the subsequent release of stress hormones. Depending on the nature of the stress, these hormones can either enhance or suppress immune functions. Our studies show an increase in the proportion of eosinophils and a decrease in lymphocytes in the blood of all experimental groups on the fifth day of drug administration. Neutrophil levels remained stable at the start of observations. By the tenth day of the experiment, however, the leukocyte profile in the blood of rats in the experimental groups had stabilized relative to their peers in the control group and relative to the previous stage of observation. A slight increase in lymphocyte content and normalization of neutrophil levels were also noted. This indicates the development of adaptive processes following the initial reaction to drug administration. It also shows that the anti-stress drug has no negative impact on the immune system's functional state.

According to other scientific sources, long-term use of anti-stress drugs can cause changes in the morphology and function of various organs and systems in laboratory animals. A study investigating the anti-stress and antidepressant activity of synthetic curcumin analogues (Hussain et al., 2022) found that they significantly increased CAT and SOD activity, moderately increased GSH activity, and demonstrated a higher antioxidant response by reducing MDA levels. Our study observed an increase in total protein levels with the use of the anti-stress drug in Group I, which may indicate the development of antioxidant reactions. A significant portion of antioxidants are protein-based, and their synthesis depends on protein metabolism.

However, there is another powerful factor that responds significantly to stress: blood glucose levels. Blood glucose levels can rise due to the release of hormones in response to stress, although this serves an adaptive purpose (Sharma et al., 2022). As noted by Santi et al. (2023), high glucose levels are possible during the use of anti-stress drugs due to their effect on hormonal regulation, as they have a direct impact on the hypothalamic-pituitary-adrenal axis. Meanwhile, a slightly elevated glucose level was recorded as early as the fifth day of observation only in rats of the control group.

The liver is the central organ regulating blood glucose levels. Therefore, we focused on the activity of liver-specific enzymes. The literature indicates that anti-stress drugs can influence the activity of organ-specific enzymes (AST and ALT) indirectly (by reducing oxidative stress and normalizing hepatocyte membranes) and that anti-stress drugs can influence the activity of hepatospecific enzymes (primarily ALT, AST, ALP, GGT) indirectly – through a reduction in oxidative stress, normalization of hepatocyte membranes, and correction of metabolic disorders. The effect depends on the drug's mechanism of action, dose, and duration of use (Zahir et al., 2021; Pillinger et al., 2025).

Administering the anti-stress drug to rats in Group III increased biomarkers of hepatic nitrosative stress and elevated liver-specific enzyme levels in serum (AST and ALT). This indicates a slight toxic effect of the drug on the animals, which remained within normal limits and gradually subsided five days after administration ceased. A similar pattern was observed in the blood serum of the control group rats compared to those in groups I and II. These changes are consistent with previous studies (Li et al., 2015; Telles-Correia et al., 2017; Zhou et al., 2017) and suggest that the preparations' chemical components possess hepatoprotective properties. These components primarily act by inhibiting lipid peroxidation, promoting liver cell membrane repair, eliminating free oxygen radicals, inhibiting mitochondrial dysfunction and the secretion of inflammatory factors, and improving cholestasis (Chalasani et al., 2021).

Thus, an analysis of our results and the literature indicates that the primary general biological mechanisms of action of the anti-stress drug are aimed at normalizing neuroendocrine regulation, reducing oxidative stress, stabilizing cell membranes, and supporting energy and protein metabolism. The aforementioned changes in internal organ mass, as well as hematological and biochemical parameters, may indicate adaptive changes in laboratory animals.

Conclusion

The experimental drug has no significant adverse effects on the general condition, hematological parameters, or macroscopic structure of the internal organs of rats and can be classified as virtually nontoxic in terms of toxicity and as low-toxicity in terms of hazard level, which justifies its use in piglets without harming their bodies.

Administration of the drug at doses of 0.15 mL per day before castration and 1.5 mL per day after castration proved to be most effective in reducing stress symptoms in piglets before and after castration, as evidenced by an increase in their live weight and growth rate. Based on the data obtained, it is advisable to recommend the specified dose for practical use in swine farming.

The authors declare no conflict of interest.

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