

The “Urticostim” Phytocomposition’s Impact on the Hematological and Immunological Status of Experimental Animals

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Abstract— An important area of veterinary biotechnology is creation of medications made of plant tissues for prevention and treatment of various animal diseases. The objective was to study the new phytocomposition “Urticostim” effect on hematological and immunological blood parameters of rats. The experimental work was carried out in accordance with the Guidelines for preclinical drug research (2012). The animals were subcutaneously injected with Urticostim phytopreparation in doses of 0.1 ml/kg, 1.0 ml/kg and 2.0 ml/kg of body weight once a day for 14 days. During the experimental period of observation, death and behavior abnormalities of animals were not noted. All the doses do not cause significant changes in the number of white blood cells and platelets and hemoglobin concentration. Doses of 0.1 ml/kg and 2.0 ml/kg of body weight contributed to a significant increase in the number of red blood cells compared with the control indices by 8% and 3%, respectively. A dose of 0.1 ml/kg caused a decrease in the bilirubin concentration of rats by 30% compared with the control indices. Daily administration of a 2.0 ml/kg dose for 14 days led to an increase in urea concentration by 20%. A dose of 0.1 ml/kg increased the absolute number of lymphocytes due to all populations of lymphoid cells. Quantitative fluctuations of T and B lymphocytes and cytotoxic T lymphocytes were characterized by an increase of 1.58, 1.66 and 1.47 times, respectively, relative to the control group indices. With an increase in the Urticostim dose by 10-20 times, a significant decrease in the concentration of T lymphocytes to 0.5 ± 0.2 thousand/ μ l was noted versus 1.2 ± 0.1 thousand/ μ l in the control index, as well as cytotoxic T lymphocytes – up to 1.2 ± 0.2 thousand/ μ l versus 1.9 ± 0.2 thousand/ μ l. The number of B lymphocytes was at the same level as the control values.

Keywords—rat blood count, phytodrug, phytotherapy, phytocomposition, hematological, biochemical, immunological indicators.

I. INTRODUCTION

One of the important areas of veterinary biotechnology is the creation of medications made of plant tissues for prevention and treatment of various animal diseases. The main advantage of herbal remedies (or phytodrugs) over synthetic drugs is a mild moderate effect on the body with the gradual development of a therapeutic effect. In addition, the phytodrugs in animal husbandry does not impose restrictions on their use [1]. In practical veterinary medicine, the expert use of phytopreparations can prevent and treat many animal

diseases, reduce livestock mortality, and reduce the cost of expensive chemotherapeutic agents. There is an opinion that phytotherapy cannot be recommended as an exceptional method of therapeutic measures; therefore, phytotherapy must be combined with other therapeutic and preventive methods and means of influencing the animal’s body, taking into account the etiology and pathogenesis of the disease. That is why one of the ways to increase the effectiveness of phytodrugs is to create phytocompositions [2, 3, 4].

Cattle postpartum diseases are some of the most common pathologies in livestock complexes that require active pharmacotherapy. In the complex treatment of retained placenta, atony, uterus hypotension and subinvolution, as well as endometritis, an important place is given to drugs with an uterotonic effect. Currently, there is a wide range of uterotonics with different mechanisms of action, but their systematic use contributes to the development of addiction and a decrease in the pharmacological effect. It is important to note that many cattle postpartum diseases occur against the background of decreased forestomach motility (hypotension and atony), which complicates the course of the initial disease. Modern uterotonics do not have a rumination effect; in connection with this it is often necessary to include rumination agents in the treatment of gynecological diseases, which increases the treatment cost. In this regard, the development of an effective drug combining uterotonic and ruminator effects with a short latent period, without restrictions on the use of animal products, is relevant and practically significant for veterinary medicine and animal husbandry in general.

At the Department of Diagnostics, Internal Non-communicable Diseases, Pharmacology, Surgery and Obstetrics of Omsk State Agrarian University, an experimental sample of the Urticostim phytocomposition was made, which includes biologically active substances with various pharmacological effects for the treatment and prevention of postpartum diseases in cows [5]. The Urticostim phytocomposition is patented well-known substances in a new galenic formulation. In order to determine the safety of a new drug before clinical use, it is necessary to conduct preclinical studies, including studying this drug’s effect on the blood system of laboratory animals, since blood is one of the main indicators that determine the functional state of the animal’s body [6].

The objective is to study the effect of the new phytocomposition “Urticostim” on hematological and immunological blood parameters of rats.

II. MATERIALS AND METHODS

The experimental work was carried out according to the Guidelines for preclinical drug research (2012) [7]. We used male laboratory white rats of Wistar breed weighing 220-230 g in the experiment, which were divided into four groups of 10 animals in each. All rats were kept in vivarium conditions and received a comprehensive rodent diet in the form of granular food, with ad libitum water from automatic drinkers. The animals were subcutaneously injected every day for 14 days with isotonic sodium chloride solution and the studied Urticostim phytopreparation in several doses. Animals of the first group served as animals with control indices; they were subcutaneously injected with isotonic sodium chloride solution at a dose of 0.5 ml/kg of body weight. The rats of the 1st experimental group were subcutaneously injected with the studied phytocomposition “Urticostim” at a dose of 0.1 ml/kg (therapeutic dose (TD) for cattle). The animals of the 2nd experimental group were subcutaneously injected with the phytocomposition “Urticostim” at a dose of 1.0 ml/kg (10 TD). Animals of the 3rd experimental group were subcutaneously injected with the phytocomposition “Urticostim” at a dose of 2.0 ml/kg (20 TD). Rat blood was taken from the jugular vein for hematological and immunological studies the day after the last drug administration. The choice of the subcutaneous administration is due to its alleged use in clinical practice. Animal manipulations were carried out in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes (Strasbourg, March 18, 1986).

Hematological parameters of rats, namely, count of white blood cells, red blood cells, platelets and hemoglobin concentration were determined using a Mindray2800Vet hematology analyzer. Some biochemical blood parameters, namely the concentration of total protein, ALT (alanine aminotransferase), total bilirubin, creatinine, urea, alkaline phosphatase were determined using a KonelabPRIME 30 ISE biochemical analyzer.

State of phagocytosis parameters was studied on the basis of evaluating the performance of oxygen-dependent microbiocidal systems of neutrophilic granulocytes in the reduction reaction of nitro-blue tetrazolium chloride (NBT test) in spontaneous (sNBT) and induced (iNBT) variants with photometric assessment of the result [8], as well as oxygen-independent mechanisms of phagocytosis according to average indicators of cytochemical lysosomal cationic test according to M. G. Shubich (1974) [9].

The population of T lymphocytes was determined in the reaction of spontaneous rosette formation with ram erythrocytes; the population of B lymphocytes was determined in the reaction of complementary rosette formation with bovine erythrocytes, which formed immune complexes with heterophilic antibodies and complement; the population of cytotoxic T lymphocytes in the reaction of indirect erythrocyte formation formed immune complexes only with heterophilic antibodies [10].

Thymus pieces were fixed in a 4% neutral formaldehyde solution, dehydrated in ascending alcohols and embedded in paraffin. Sections of 3-5 microns were obtained on a LabCut

4055 rotary microtome (Slee, Germany), stained with hematoxylin and eosin. Microphotography of histological preparations was carried out on the Altami BIO 1T microscope with a UCMOS0300KPA digital ocular camera.

Digital data were processed using the Statistica 10 program and presented as arithmetic mean (M) and mean error (m). The reliability of the results was determined using Student's t test. The difference between the experimental groups was considered statistically significant at $p < 0.05$.

III. RESEARCH RESULTS

Animal mortality was not recorded throughout the entire experimental period; rats were active and they consumed food and water well. The fur was shiny. The injection spots were without signs of inflammation: redness, soreness, and swelling were not noted. Fecal boluses in rats of the experimental groups had a dense consistency, without foreign inclusions, externally did not differ from the animals of the control group. The number of bowel movements in all experimental animals had no significant differences.

With the postmortem autopsy in most animals of the experimental groups, macroscopic changes in the internal organs were not observed. The liver was of normal size, not enlarged, the edges were sharp, the surface of the organ was shiny, dark brown in color without hemorrhage, and the scraping was not abundant in the section. In two rats of the third experimental group, lighter shades of the liver were recorded. The kidneys in all animals of the experimental groups were of normal size, not enlarged, dark brown in color, the capsule from the organ was easily removed, and the border of the cortex and medulla was preserved in the section. Rats' hearts were also of normal size, not enlarged, the apex of the heart was sharp, the coronary vessels were desolate, and no hemorrhages were recorded. The lungs of all animals were pale pink, without hemorrhage. The stomachs were not enlarged and contained a small amount of fodder masses; the gastric mucosa was without macroscopic lesions, pale gray. The intestines also did not have macroscopic changes, Peyer's patches were well visualized, the mucous membrane along the entire intestine without damage and hemorrhage. The mesenteric lymph nodes were without external changes and not enlarged. The spleens are dark cherry in color, not enlarged, the edges are sharp, and the scraping in the section is not plentiful. Results of the study of hematological parameters of rats are presented in Table 1.

TABLE I. HEMATOLOGICAL BLOOD PARAMETERS OF EXPERIMENTAL ANIMALS

Group	Parameter			
	WBC	RBC	H	P
Control indices	19.7±3.7	8.4±0.1	143.3±2.2	803.3±75.5
Experiment 1, dose 0.1 ml/kg	20.2±0.3	9.1±0.2 ^a	149.7±3.3	761.3±60.8
Experiment 2, dose 1.0 ml/kg	18.9±2.3	8.9±0.3	140.5±1.5	847.0±181.0
Experiment 3, dose 2.0 ml/kg	12.1±1.1	8.7±0.1 ^a	150.0±1.0	737.3±143.4

^a $p \leq 0.05$. WBC - white blood cells, 10⁹/l; RBC - red blood cells, 10¹²/l; H - hemoglobin, g/l; P - platelets, 10⁹/l.

Table 1 shows no reliable changes in the indicators of white blood cells, platelets and hemoglobin concentration in animals of all experimental groups. The red blood cells count was significantly increased in rats of the 1 and 3 experimental groups compared with the control group by 8% and 3%, respectively, which may be due to the release of red blood

cells from the depots organs of the experimental groups animals, or stimulation of hematopoiesis, and regarded as physiological adaptive anti-stress reactions of increased activation developing in response to small acting factors (Garkavi L.Kh., Kvakina E.B., 1990, 1996).

Proteins play a crucial role in maintaining the homeostasis, being the basis for building body cells, hormones, enzymes, antibodies and other formations that perform various functions in the body. A change in the concentration of protein in the blood serum indicates a change in the functioning of morphofunctional systems, primarily digestive and excretory organs. The effect of the experimental sample of the Urticostim phytocomposition on protein metabolism was judged by the concentration of total protein, creatinine and urea – the final products of protein metabolism. Hepatotoxicity was excluded by assessing changes in the concentration of ALT, total bilirubin and alkaline phosphatase, and nephrotoxicity – by changing the concentration of creatinine. Results of biochemical blood parameters of rats are presented in Table 2.

TABLE II. BIOCHEMICAL BLOOD PARAMETERS OF EXPERIMENTAL ANIMALS

Group	Parameter					
	TP	ALT	TB	Cr	U	Aph
Control indices	72.5 ±1.8	65.0 ± 6.2	2.3± 0.3	37.5 ±1.5	6.4± 0.6	378.5 ±36.5
Experiment 1	75.2 ±1.3	55.2 ± 7.6	1.6± 0.4 ^a	38.8 ±2.4	6.9± 1.3	408.8 ±41.9
Experiment 2	77.5 ±1.7	65.0 ± 7.1	2.3± 0.4	38.5 ±3.7	6.6± 1.2	589.3 ±83.4
Experiment 3	76.6 ±1.2	60.3 ± 12.4	2.1± 0.1	35.6 ±2.6	7.7± 2.0 ^a	467.7 ±178.4

^a p≤0.05. TP – total protein, g/l; ALT – alanine aminotransferase, mmol/l; TB – total bilirubin, μmol/l; Cr – creatinine, μmol/l; U – urea, mmol/l; Aph – alkaline phosphatase, units/l.

Results of the study of biochemical blood parameters of experimental groups indicate the absence of significant deviations in the concentration of total protein in all experimental groups, with a tendency to increase this indicator against the background of normal ALT values. This condition is possibly due to an improvement in the assimilation of protein supplied with food, since biologically active substances that enhance the secretion of glands are included in the phytocomposition.

A decrease in bilirubin concentration by 30% was observed in blood of rats of the 1 experimental group, compared with the control group of animals (p <0.05). Bilirubin is formed in the reticulo-endothelial system under the action of the enzyme biliverdin reductase from biliverdin, which is the product of the breakdown of heme and the destruction of red blood cells. A decrease in this indicator in rats treated with the Urticostim phytocomposition at a dose of 0.1 ml/kg for 14 days is probably due to the influence on the factors that regulate the process of erythrocyte destruction. It was in animals of this group that an increase in the number of red blood cells was noted. It is believed that circulating bilirubin is involved in the protection of human tissues from lipid peroxidation [11]. However, the mechanisms causing this phenomenon currently are not understood. It is well known that activation of the processes of formation of free oxygen and peroxide radicals occurs in numerous pathological conditions. Moreover, it is believed that a decrease in blood bilirubin level may be a significant marker for assessing the

overall antioxidant status of a human or animal organism [11]. The nephrotoxic effect of high doses of phytocomposition is indicated by an increase in blood urea concentration. Thus, in animals of the 3 experimental group receiving the Urticostim phytocomposition at a dose of 2.0 ml/kg for 14 days, an increase in urea concentration in comparison with the control group by 20% (p <0.05) was noted, which may be caused by a violation of the filtering ability of the kidneys. There were no statistically significant changes in the concentration of total protein, ALT, creatinine and alkaline phosphatase in animals of all experimental groups compared with the control indices.

The immune system is a fast-response system for introduction of any foreign agent. Medicinal substances are no exception. Changes in the parameters of the immune response reflect the course of the adaptation period in animals and allow us to understand the mechanism of the immunotropic effect of the tested substances.

An analysis of the immunological parameters presented in Table 3 showed that the introduction of the phytocomposition “Urticostim” for 14 days in a therapeutic dose (0.1 ml/kg) contributed to an increase in the absolute number of lymphocytes, which occurred due to all populations of lymphoid cells, although, the indicator was not reliable. The quantitative fluctuations of T, B lymphocytes, and cytotoxic T lymphocytes, although they were characterized by an increase of 1.58, 1.66, and 1.47 times, respectively, relative to the indices of the control group, nevertheless, were also not statistically significant. Slight fluctuations of lymphoid cells in the direction of their increase against the background of a two-week administration of the tested phytocomposition is a consequence of activation of detoxification mechanisms aimed at removing the drug from the body.

With an increase in the dose of the phytocomposition “Urticostim” by 10-20 times (1.0 ml and 2.0 m /kg), on the contrary, a significant decrease in the concentration of T lymphocytes to 0.5±0.2 thousand/μl was noted against 1.2±0.1 thousand/μl (P<0.05) in the control, as well as cytotoxic T lymphocytes up to 1.2±0.2 thousand/μl against 1.9±0.2 thousand/μl (P<0.05). The number of B lymphocytes was approximately at the same level.

Inhibition of the T cell link of the immune response of the tested phytocomposition, administered in toxic doses, is considered as a negative effect, which must be taken into account in the further study of the drug.

TABLE III. IMMUNOLOGICAL BLOOD PARAMETERS OF RATS OF EXPERIMENTAL GROUPS

Parameter	Groups			
	Control	Experiment 1	Experiment 2	Experiment 3
Lph	7.16±0.6	9.0±2.1	8.1±2.0	8.0±1.1
Nph	2.8±0.2	2.3±0.4	2.0±0.3	2.1±0.3
T Lph	1.2±0.1	1.9±0.5	0.5±0.2*	0.5±0.2*
B Lph	1.2±0.1	2.0±0.5	1.3±0.2	1.3±0.3
CTL	1.9±0.2	2.8±0.6	1.2±0.2*	1.2±0.2*
CCC	1.2±0.09	1.2±0.1	1.1±0.3	1.1±0.07
sNBT	1.2±0.03	1.2±0.03	1.1±0.01	1.1±0.04
iNBT	1.2±0.04	1.2±0.02	1.2±0.02	1.2±0.01

^a p≤0.05. Lph – lymphocytes, thousand/ μ l; Nph – neutrophils, thousand/ μ l; T Lph – T lymphocytes, thousand/ μ l; B Lph – B lymphocytes, thousand/ μ l; CTL – cytotoxic T lymphocytes, thousand/ μ l; CCC – average cytochemical coefficient of lysosomal cationic protein; sNBT and iNBT – in optical density units.

It should be noted that the studied drug did not affect the state of both oxygen-dependent and oxygen-independent systems of neutrophilic granulocytes, although with an increase in the dose of the Urticostim phytocomposition (10-20 TD), a tendency to a decrease in the bactericidal activity of leukocytes was noted.

The severity of the body's defensive reactions to the influence of external factors largely depends on the morphological and functional state of the thymus, the central organ of immunogenesis responsible for the differentiation and cloning of T Lph. Microscopic examination of the rats' thymus indicates that in all animals of the control (isotonic sodium chloride solution at a dose of 0.5 ml/kg) and the 1 experimental (Urticostim at a dose of 0.1 ml/kg, which is a cattle therapeutic dose (TD)) groups the thymus had a well-defined connective tissue capsule with the septa that divided the parenchyma into lobules. Each lobule consisted of peripheral cortical and central cerebral zones. The main criterion for this separation was the density distribution of thymocytes populated in these sections of lobules. The cortical zone occupied the main area of the entire lobule and was characterized by a dense distribution of thymocytes. The cerebral zone was characterized by a low density of distribution of lymphocytes and occupied a relatively small area of the entire lobule. Single macrophages around blood vessels were detected. Microscopic examination of the thymus in animals of the 2 experimental (Urticostim at a dose of 1.0 ml/kg (10 TD)) and 3 experimental (Urticostim at a dose of 2.0 ml/kg (20 TD)) groups was recorded less dense distribution of thymocytes, i.e. enlightenment (sparseness) of the cortical zone in comparison with the thymus of animals of the control group, as well as the formation of individual Hassall's corpuscles in the brain substance, which indicates atrophic processes in the organ. It is possible that thymic atrophy is transient in response to the effects of excessive doses of phytocomposition, and unlike age-related involution, stress-induced atrophy will be accompanied by subsequent organ restoration.

IV. CONCLUSION

Results of the experimental study indicate that subcutaneous administration of the new phytocomposition "Urticostim" at a dose of 0.1 ml/kg in 14 days does not cause negative changes in the morphological, biochemical and immunological blood parameters and has a "mild" immunostimulating effect in laboratory animals. A two-week subcutaneous administration of the Urticostim

phytocomposition at a dose of 2.0 ml/kg promotes the development of iatrogenic nephropathy and immunosuppression of the cellular component of the immune response in rats.

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