



Studying the Safety of the Antiviral Preparation Triazavirin

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ABSTRACT: In the modern conditions of the development of veterinary medicine, the prevention and control of viral agents causing outbreaks of infections and significant economic damage to agriculture are of great importance. Of interest are new antiviral drugs, both veterinary and medical. Before the introduction of a new medicinal product into production, its testing and certification based on several indicators are necessary. In the present study, the effect of the preparation Triazavirin on the hematological, biochemical and immunological parameters of mice blood is considered. For the experiment, outbred albino mice were used. The care and feeding of different experimental groups of animals was identical and followed the guidelines for keeping laboratory animals in the vivarium of research institutes and educational institutions. The preparation was fed to the mice at the dosage of 0.00025 g (0.25 mg) per animal for five days. The killing of the experimental mice was performed on the 6th, 15th and 30th days. Throughout the experiment (30 days), the animals were observed and indicators, such as general condition, behavior, death, and signs of intoxication were, evaluated. During the experiment, hematological, biochemical, and immunological parameters were investigated as well. The possible toxic effect of the preparation on the experimental animals has been studied. As a result of the experiment, behavior, appetite, autogrooming, hair coat, coordination of movements, urination, and defecation have not encountered serious changes. Analysis of hematological indicators revealed leukocytosis on the sixth day of the experiment; however, by the 30th day of the experiment, the leukocytes count had returned to the normal values. A decreased level of hormones was observed after administering the preparation. It is shown that Triazavirin in the therapeutic dosages does not have pronounced distant toxic effects.

Keywords: hematological, biochemical and hormonal blood indicators, laboratory mice, antiviral drug, Triazavirin.

I. INTRODUCTION

Modern medicine can provide a significant list of antiviral and immunostimulating drugs. Unfortunately, in veterinary practice, antiviral drugs are rarely used to treat animal viral infections. Significant progress has been made in viral chemotherapy, but many viruses mutate and develop resistance to some commonly used drugs. The search for new treatments continues [1-4].

Plant materials contain a huge number of biologically active compounds that allow developing new antibacterial and antiviral chemotherapy drugs. Different parts of plants and components, as well as terpenoids, are used. For example, birch bark is used to receive a triterpene betulin, having an antiviral effect. Saponin-containing plants, belonging to different families, are studied: Fabaceae, Sapindaceae, Caryophyllaceae, Betullaceae, Berberidaceae, Hippocastanaceae, Chenopodiaceae, Araliaceae. Preliminary studies show that preparations obtained from plants *Acacia concinna* (Fabaceae), *Sapindus mucorossi* (Sapindaceae), *Allochrusagypsophiloides* (Caryophyllaceae) and *Saponaria officinalis* (Caryophyllaceae) have antiviral properties [5-8].

A variety of modern methods for the synthesis of antibacterial components are actively applied. Nanotechnology is one of the most promising areas in

pharmacology and metal nanopowders and other types of nanomaterials are widely studied in many industries.

The study of the antibacterial and antiviral properties of nanoparticles is a priority. Silver nanoparticles with sizes of 25, 80, and 130 nm and their antibacterial, antiviral, and antifungal properties, used both in medicine and in veterinary medicine, are studied. The properties of iron are investigated. For the synthesis of iron nanoparticles, seeds of plants *Manilkara Zapota* (Chickoo) are used [9, 10].

Significant advances in biology, including the discovery of DNA and RNA, allow for a different look at the nature of the virus and approaches to the treatment of viral diseases. The study of the nucleic bases of DNA and RNA and their analogues, nucleosides, allowed developing a number of antiviral drugs, such as acyclovir and ribavirin. Further developments made it possible to create a number of azoloazines – structural analogues of purine bases of DNA and RNA, serving as the basis for new antiviral drugs. A new family of antiviral drugs has been created: pyrazolo-, imidazolo-, 1,2,4-triazolo[5,1-c]-, 2,4-triazine-7 (4H) (1) and 7-aminoazolo [5,1-c]-1,2,4-triazines (2). The first drug created on the basis of compounds of this class is Triazavirin (sodium salt of 2-methylthio-6-nitro-1, 2, 4-triazolo [5, 1-s]-1, 2, 4-triazine-7 dihydrate) [7, 11].

At present, new preparations are being introduced in large amounts into the veterinary sector of agriculture. Chemotherapeutic, antibacterial, and immunostimulant preparations are in the greatest demand. Preparations from the antiviral group are required but used insignificantly [1-3].

In veterinary practice, work is underway to introduce antiviral drugs. However, there are significant difficulties in administering drugs to animals and in mass mode. In particular, the injection form of the Arbidol preparation is being developed and used in industrial pig breeding as an agent with immunostimulating and antiviral effects [1-6, 12-13].

Tests of new dosage forms include comprehensive consideration of the preparation's properties during the introduction and standardization. Studies are conducted on the effects of chemotherapeutic compounds in vitro and in vivo on cell cultures and different experimental laboratory animal models in the conditions of an epizootological situation [1-5, 14].

Determination of acute and chronic toxicity, irritant and allergic effects, carcinogenic and mutagenic properties, and immunotoxicity is a mandatory phase during the introduction of new medications. Determination of the cumulative properties and analysis of late effects become relevant [4, 12, 15].

Studying the hematological and biochemical changes in the blood is the quickest and the most indicative method of studying toxicological long-term effects of a preparation on laboratory animals in the conditions of a complex, actively interacting biological system. In veterinary medicine, the specific features of biological reactions to the use of certain chemical compounds are also very relevant, which also requires certain tests. A blood test for hematological, biochemical, hormonal indicators is one of the available and highly indicative methods [11, 13, 14, 16].

II. MATERIALS AND METHODS

The study was performed at the Interdepartmental Educational-and-Scientific Laboratory of Biotechnology of the Federal State Budget Institution of Higher Education "Izhevsk State Agricultural Academy". For the

experiment, laboratory outbred white mice were used that were in the vivarium of the Department of Physiology and Zoohygiene of the Department of Veterinary Medicine [11, 14, 16].

Laboratory mice were kept in specialized cages with drinkers; the hygienic conditions in the vivarium were in line with the established norms. Care and feeding of different experimental groups of animals were identical and corresponded to the methodical recommendations for care of laboratory animals in vivariums of research institutes and educational institutions. 80 mice were used in the experiments. Groups of animals were chosen by the method of random sampling. The references were 20 mice that were slaughtered by decapitation before the introduction of the preparation. The remaining 60 mice received Triazavirin intragastrically. The preparation is ethical; however, its numerous toxicological characteristics have not been studied. The preparation was administered daily for five days, once a day at the dosage of 0.00025 g (0.25 mg). The experimental mice were slaughtered on day 6, 15, and 30.

Throughout the experiment that lasted 30 days, the animals were observed, and indicators such as overall condition, behavior, death, symptoms of toxicity were assessed (Table 1). During the experiment, hematological, biochemical, and immunological parameters were also studied (Tables 2, 3 and 4).

Hematological studies were performed using an automated hematological analyzer BC-2800Vet; the biochemical studies – using an automatic biochemical analyzer Mindray BS-300; hormones – using an automatic enzyme immunoassay analyzer Alisei (SEAC srl); and immunological studies – using a biochemical analyzer Clima MC-15 RAL.

Statistical analysis was performed by the method of variation statistics using the Student's t-test [1].

III. RESULTS

Intragastrical administration of Triazavirin to the laboratory mice was performed in a dosage of 0.25 mg per animal. Several changes in blood indicators were observed (Table 2).

Table 1: Scheme of the experiment.

Experiment No.	Dosage	Number of animals	Mode of introduction	Dose schedule
	0.00025 g of the preparation dissolved in 0.2 ml of normal saline solution	60 mice	Intragastrically	Once a day, for five days
	Reference group	20 mice		The preparation was not administered

Table 2: The dynamics of mice blood hematological parameters in the experimental and the reference groups.

Indicator	Experiment duration			
	Before the experiment	Day 6	Day 15	Day 30
Leukocytes, *10 ⁹ /l	14.21 ± 0.5	15.63 ± 0.02**	7.075 ± 0.709313	11.875 ± 0.803286
Lymphocytes, %	60.3 ± 0.027	58.31 ± 0.76**	51.225 ± 3.138571	61.625 ± 4.235132
Monocytes, %	4.39 ± 0.2	3.71 ± 2.161*	3.542857 ± 0.484452	5.325 ± 1.232702
Granulocytes, %	35.64 ± 0.079	36.22 ± 0.19**	44.425 ± 2.849796	33.05 ± 3.249231
Erythrocytes, *10 ¹² /l	9.04 ± 0.083	9.31 ± 0.05499**	9.3825 ± 0.153748	9.33 ± 0.259009
Hemoglobin, g/l	126.4 ± 0.09	128.25 ± 0.9*	128.625 ± 4.140124	128.375 ± 4.131488
Hematocrit, %	35.13 ± 0.87	37.35 ± 0.53*	36.375 ± 1.123888	36.85 ± 1.186381
Platelets, *10 ⁹ l	756.86 ± 40.47	1041.75 ± 83**	940.625 ± 104.802	820.25 ± 75.75919

* – P ≥ 0.950, ** – P ≥ 0.990, *** – P ≥ 0.999

During the analysis of the hematological data of red blood, no sharp fluctuations from the normal values and the reference group were observed. The level of erythrocytes throughout the entire experiment after the preparation administration increased on average by 3.3 %. During the experiment, hemoglobin increased on average by 1.1 %, hematocrit – by 4.9 %, platelets by day six increased by 37.6 %, by day 15 and day 30 – by 24.3 % and 8.4 %, respectively.

On day six after the preparation administration, an increased level of leucocytes by 39.43 % was observed; on day 15, this indicator decreased sharply by 36.89 %, compared to the reference, and by day 30, the leukocyte count normalized and exceeded the reference by as little as 5.9% (Table 3).

In considering an indicator such as lymphocyte count, a strong trend to reduction up to day 30 of the experiment

was observed. By day six, the leukocyte count decreased to 96.7% (by 3.3%), by day 15 – to 84.95% (by 15,05 %), and only by day 30, the authors observed the normalization of this indicator and its growth to 102.2%.

Monocytes also sustain certain changes during the administration of the preparation. By the end of the treatment period (six days), the indicator dropped to 84.5 % (by 15.5 %), compared to the reference, by day 15 of the experiment – to 80.6 % (by 19.4 %), by day 30 the monocytes count normalized – 121, 3%, and even exceeded the reference level. By day six, granulocytes count increased by 1.6%, by day 15 – by 24.65%, by day 30 – it decreased to 7.3%, compared to the reference (Table 2).

Analysis of the biochemical parameters of mice blood serum revealed a number of regularities (Table 3).

Table 3: Dynamics of the biochemical parameters of mice blood serum experimental and control groups.

Indicator	Duration of the experiment			
	Before the experiment	Day 6	Day 15	Day 30
Cholesterol, mmol/l	2.4 ± 0.03	2.34 ± 0.00225*	2.2625 ± 0.187023	2.6375 ± 0.105115
Total protein, g/l	63.71 ± 1.4	68.59 ± 1.905*	70.775 ± 1.526288	71.5625 ± 3.434486
Albumin, g/l	38.61 ± 0.7	44.49 ± 2.3*	41.8 ± 1.158663	42.18571 ± 0.978198
Globulins, g/l	25.14 ± 0.35	24.1 ± 0.09**	28.975 ± 1.12516	26.625 ± 1.781728
Albumin-globulin ratio	1.55 ± 0.06	1.85 ± 0.14*	1.46125 ± 0.0797	1.74625 ± 0.157808
Creatinine, μ mol/l	29.14 ± 2.3	23.57 ± 1.5*	43 ± 14.06744	26.14286 ± 1.370238
ASAT, units/l	266 ± 3	256 ± 2.265**	213 ± 13.84609	237.75 ± 25.45076
ALAT, u/l	33.25 ± 0.644	31 ± 0.41**	23.625 ± 4.566718	22 ± 4.386125
Urea, mmol/l	5.56 ± 0.09	4.64 ± 0.331**	5.785714 ± 0.247298	4.514286 ± 0.231382
Alkaline phosphatase, units/l	150.25 ± 2.8	157 ± 1.03*	135.5 ± 17.248667	119.8 ± 16.73738

* - P ≥ 0.950, ** - P ≥ 0.990, *** - P ≥ 0.999

The level of cholesterol during the experiment after the preparation administration varied insignificantly at the bottom limit of the physiological norm. The level of ALAT gradually decreased by day 30 of the experiment, by day six – by 6.77 %, by day 15 – by 28.9%, and by day 30 – by 33.83%. The level of ASAT in the reference group was close to the upper limit of the norm; during the preparation administration this indicator decreased by day six by 3.76%, by day 15 – by 19.9%, and by day 30 – by 10.6%, compared to the reference.

By day six of the experiment, alkaline phosphatase increased by 4.5%, after which it decreased by day 15 by 9.8%, and by day 30 – by 20.3%.

The level of urea by day six of the experiment decreased by 16.55%; by day 15, an increase only by 4.1% was observed, and by day 30, the level of urea increased by 18.8%.

The level of creatinine by day six of the experiment increased by 11.5%; by day 15 of the experiment, a significant increase in this indicator to 103.41% was observed; and by day 30, stabilization and increase by only 23.65%, compared to the reference, were observed.

During the experiment, the total protein by day 30 increased to the upper limit of the physiological norm. By day six, protein level increased by 7.66%, by day 15 – by 11.1%, and by day 30 – by 12.32%, compared to the reference.

The albumin fraction of proteins by day six of the research increased by 15.2%, by day 15, it decreased, and excess over the reference was only 8.3%, and by day 30, the increase was 9.3%.

The globulin fraction of proteins also changed; by day six of the experiment, the level of globulins decreased by 4.1 %, by day 15, the fraction increased by 15.3% and by day 30 – by 5.9%, compared to the reference.

Changing the ratio of the albumin-globulin fraction at various periods of the experiment also entails fluctuations of the respective coefficient. By day six, the albumin-globulin ratio increased by 19.3%, by day 15, it decreased by 5.7%, and by day 30, the increase was equal to 12.7%.

Analysis of the hormonal activity of the experimental mice revealed changes in the indicators of a number of hormones (Table 4).

Table 4: The concentration of hormones in mice blood serum experimental and control groups.

Indicator	Experiment duration			
	Before the experiment	Day 6	Day 15	Day 30
Thyrotropin, μIU/ml	0.17 ± 0.00135	0.16 ± 0.0047*	0.305 ± 0.025	0.2
T ₄ , pmol/l	15.4 ± 0.0055	15.2 ± 0.1*	19.13 ± 0.12	11.35 ± 0.25
T ₃ , nmol/l	1.4 ± 0.04378	1.26 ± 0.04509*	1.59 ± 0.61	0.91 ± 0.19
Cortisol, nmol/l	21.3 ± 0.58103	42.8 ± 8.1**	79.5 ± 4.5	54.45 ± 10.05

* - P ≥ 0.950, ** - P ≥ 0.990, *** - P ≥ 0.999

By day six of the experiment, Thyrotropin decreased by 5.9 %; by day 15, it increased by 79.4 %, and by day 30 – by 17.65 %. Hormones T3 and T4 that are typically related to Thyrotropin also experienced changes.

For instance, the level of thyroxine by day six of the experiment decreased by 1.3 %, by day 15 – increased by 24.2 %, and by day 30, a decrease by 26.3 % was noted, compared to the reference.

T3 by day six of the experiment decreased by 10 %, by day 15 – increased by 13.6 %, and a sharp drop by 35 % was noted by day 30.

The level of cortisol during the experiment naturally increased: by day six – by 100.9 %, by day 15 – by 273.2 %, and by day 30 – by 155.6 %.

IV. DISCUSSION

The observation during 30 days revealed no significant abnormalities in the clinical state of health of the mice. Behavior, appetite, thirst, autogrooming, state of the hair coat, movement coordination, urination, defecation, compared to intact animals, did not undergo significant changes.

Analysis of hematological indicators revealed leukocytosis on the sixth day of the experiment; however, by the 30th day of the experiment, the leukocyte count returned to the reference values.

The increased platelets count was observed on day six of the experiment. By day 30, the platelets count decreased. This suggests the formation of sludge complexes in the blood vessels of the kidneys and the liver [15]. It should also be noted that despite the fluctuations, this indicator did not go beyond the physiological norm.

Analyzing the parameters, one can draw a conclusion about the absence of symptoms of intoxication and anemia; hemoglobin level and erythrocytes count did not significantly fluctuate during the experiment.

When analyzing biochemical parameters, several changes were observed. The indicators that reflect the state of the hepatobiliary system and cardiotoxicity are within the physiological norm. Cholesterol and alkaline phosphatase reflect the state of the biliary structure of the liver, and their increase is evidence of cholestasis, which occurs naturally with increasing the load on a particular organ during the administration of the preparation.

By day 15, creatinine in the experimental group of animals increased by 103.41 %. The indicator reflects the intensive activity of the urinary system, kidneys in particular, at this stage of the experiment. By day 30, the indicator normalized within the physiological norm.

Throughout the experiment, an increased level of total protein was observed. A similar blood picture is characteristic of stabilization of the hepatic cell system, and of activated synthetic functions of the liver.

Analyzing the ratio of proteins in the albumin-globulin fractions, the fluctuations in the level of albumin and globulin within the physiological norm were observed for five days after the preparation administration. Fluctuations in the albumin-globulin coefficient cannot be a reliable indicator of the presence of an inflammatory reaction after taking the experimental drug.

The pituitary hormone (Thyrotropin) and the hormone of the thyroid gland (T3, T4) are in direct and immediate

interaction. A sharp decrease in the level of hormones was observed after five days of treatment with the experimental preparation. Growth and the simultaneous increase in these indicators by day 15 of the experiment is an evidence of stress effect of the preparation on the hypothalamic-pituitary system, which reflexively triggers the thyroid and adrenal glands. By day 30 of the experiment, Thyrotropin stabilized, but due to the increase on day six of the experiment, thyroid hormones T3 and T4 had not had time to sync.

Cortisol is a stress hormone, and its increase during the experiment is caused by its conditions – the presence of a stressful situation (fixation of mice, intragastric administration), and the introduction of the preparation and activation of the pituitary gland. As a result, the authors observed an increase in the cortisol level by day six of the experiment, the peak of growth occurred on day 15, and the gradual decrease – by day 30 of the experiment.

In general, the research has been performed in accordance with the recommendations for assessing the safety of preparations, and the results coincide with the data of other researchers [11, 14, 16].

Thus, the research aimed at assessing the safety of the ethical preparation Triazavirin has shown that the preparation in the recommended dosages is harmless, and does not have a pronounced toxic effect on laboratory mice.

V. CONCLUSION

– Studying the safety of the antiviral preparation Triazavirin on mice has shown the good state of health of the experimental animals. Mice behavior was characterized by stability; no deviations from the norm were observed.

– During the experiment, the hematological parameters remained within the limits of the physiological norm; however, slight leukocytosis and increased platelet count were observed on day six of the experiment.

– The biochemical status was characterized by an increased level of creatinine, which indicated the increased functional load on the organs of the urinary system during the experiment.

– The hormonal status was characterized by significant changes– cortisol, Thyrotropin, T3, T4. Therefore, the hypothalamic-pituitary system actively monitors and triggers compensatory and adaptive mechanisms, which results in the activation of the thyroid and the adrenal glands.

Conflict of interest. The authors declare that there is no conflict of interest to disclose.

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