Sub-acute injection toxicity of Florfenicol VS 30

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Abstract. This study is aimed at identifying organs and/or target organs systems that are most sensitive to the toxic effect of the drug under study, as well as determining the level of cumulation and reversibility of pathological changes in the animal body influenced by the pharmacological substance. During a 14-day sub-acute (intramuscular injection) experiment on outbred rats, it has been found that doses of 2.260 mg/kg (1/5 of LD50=1.1300 mg/kg) and 1130 mg/kg (1/10 of LD50=1.1300 mg/kg) were toxic and the dose of 565 mg/kg was threshold (1/20 of LD50=1.1300 mg/kg). The investigated product Florfenicol VS 30 (developed by VETSFERA, Russia) in the tested doses caused a significant decrease in the body weight and significant changes in hematological and biochemical blood parameters. Integral indices in test groups were characterized by severe depression, decreased food activity, weakened skeletal muscle tone, and poor skin turgor. Gastrointestinal disorders were observed during all weight recording periods, while animals were injected with toxic doses. By day 14 of the experiment, the drug at a dose of 565 mg/kg (tolerable level) caused no changes in the behavior of experimental rats or their functional states. The disturbed hemopoiesis and significant hypoproteinemia revealed were reversible.

1. Introduction
Florfenicol VS 30 is an antibacterial drug of the fenicol group intended for the treatment of bacterial diseases of pigs and cattle; it is produced in the form of a solution for injection and contains 300 mg of florfenicol in 1.0 ml. Florfenicol is a broad-spectrum antibacterial agent to treat bacterial diseases in veterinary practice [1].

New drugs and their treatment doses implemented into clinical practice presuppose high levels of efficacy and safety of pharmacological substances, proven in accordance with modern requirements. For this purpose, the scientific research complied with the regulated procedure at the stage of laboratory and experimental (preclinical) procedures for general toxic properties and specific pharmacological activity to be assessed [2].
2. Materials and methods
To determine the sub-acute toxicity parameters of laboratory animals was a necessary stage of the research to assess the safety and study a medicinal product intended for veterinary use in preclinical and clinical studies [3, 4, 5, 6].

The animals were treated in accordance with the requirements of GOST 33044-2014 “Principles of Good Laboratory Practice,” 2015; Order of the Ministry of Health of the Russian Federation No. 199н dated March 1, 2016 “On Approval of the Rules of Good Laboratory Practice” and guidelines for preclinical studies of the Scientific Centre for Expert Evaluation of Medicinal Products; and the Federal Law No. 61-FZ of April 12, 2010 “On the Circulation of Medicines” (as amended on December 28, 2017).

The study was conducted at the International Research Center for the Protection of Human, Animal Health and Environment (OOO IRC “OZOS”). Manipulations with animals were carried out in the vivarium of the VNIIP named after K.I. Scriabin.

The sub-acute toxicity of Florfenicol VS 30 was tested on 40 white outbred male rats, weighing 263-333 g. Three Test groups and one Control group were formed, 10 animals each.

The experimental animals were kept in a vivarium in accordance with the requirements specified in the relevant reference documentation [7, 8]. Proper microclimate parameters were maintained in the room where the laboratory rats were kept [9]. The animals were fed with a complete extruded compound feed for laboratory animals; drinking was performed ad libitum with prepared water from standard drinkers.

Rats were injected intramuscularly (posterior femoral muscle set) with the drug under study in the form of a provided solution to be not diluted for 14 days daily, alternating the injection areas.

The doses of 2.260 mg/kg (1/5 of LD50=1.1300 mg/kg), 1130 mg/kg (1/10 of LD50=1.1300 mg/kg), and 565 mg/kg (1/20 of LD50=1.1300 mg/kg) were tested. The LD50 value was obtained in previous studies and calculated by the Kerber’s method. The drug was in a volume of 0.20, 0.10, and 0.05 ml per 100 g of the animal weight; the density was 1.13 g/cm$^3$. The animals in Control group were injected with water for injection (OOO Groteks, series 971117, EXP December, 2020) in a volume of 0.2 ml per 100 g.

The chosen doses for a sub-acute experiment met the requirements in the guidelines for assessing sub-acute toxicity [10]. The research design is shown in table 1.

Table 1. Research design of sub-acute toxicity of Florfenicol VS 30 on outbred rats.

| Group № | Species and gender | n | Drug | Drug dose, mg/kg | Drug volume, ml | Dose schedule |
|---------|--------------------|---|------|-----------------|----------------|---------------|
| 1       | Rats ♂              | 10| Florfenicol VS 30 | 2 260           | 0.20/100 g     | Intramuscularly, daily, once a day within 14 days |
| 2       | Rats ♂              | 10| Florfenicol VS 30 | 1 130           | 0.10/100 g     | Intramuscularly, daily, once a day within 14 days |
| 3       | Rats ♂              | 10| Florfenicol VS 30 | 565             | 0.05/100 g     | Intramuscularly, daily, once a day within 14 days |
| 4       | Rats ♂              | 10| Water for injection | -               | 0.20/100 g     | Intramuscularly, daily, once a day within 14 days |

Note: n is the number of animals in the group; ♂ means males.

The animals’ performance statuses, behavior, and possible deaths, as well as manifestation of intoxication symptoms were being monitored for 14 days. The animals’ body weight dynamics was assessed on days 7 and 14 of the experiment [2, 10].

On the day after the last injection, half of the animals in each group were euthanized and blood samples were taken to determine hematological and biochemical parameters; these manipulations were
repeated 10 days later [12]. On days 1 and 10 after the drug course, an autopsy of experimental animals was performed. The subcutaneous tissue, liver, lungs, kidneys, heart, spleen, stomach, large and small intestines, as well as muscle tissue in the injection areas were grossly examined. Before terminal procedures, the animals were deprived of food and then weighed. The rat weight values were used for the subsequent calculation of the mass coefficients of organs (liver, kidneys, spleen, lungs, and heart). The functional state of the central nervous system (CNS) was assessed visually by observing motor activity and reactions to external stimuli [11].

3. Research results

After Florfenicol VS 30 at a dose of 2.260 mg/kg was injected, 8 deaths (out of 10 rats) was recorded in Test group I during 11 days of observation. The intoxication pattern was characterized by cachexia, general depression, and apathy. All rodents in this group had diarrhea, decreased appetite and water consumption, weakening of skeletal muscle tone, and skin turgor.

The drug at a dose of 1.130 mg/kg did not cause the death of animals during the period of intramuscular injections, but by the 6th day after the discontinuation of the drug, 2 rats out of 10 animals in Test group II died. The intoxication signs were similar to Test group I. The Test group II animals were emaciated and apathetic.

The dose of 565 mg/kg also contributed to a significant decrease in the animals’ body weight, weakening of their skin turgor, gastrointestinal disorders, and general depression. The death of animals in Test group III was not registered. The body weight dynamics of experimental animals is shown in figure 1.

![Figure 1. The body weight dynamics of experimental rats.](image)

The postmortem picture of the dead animals – autopsy was characterized by gastric flatulence and sharp blood filling of the vessels of the intestinal walls and mesentery.

On days 1 and 10 after the withdrawal of the drug injections in the experimental groups, a significant increase in the mass coefficients of the liver was noted. There were no changes in the gross appearance of the organ, so the significance of the liver weight in Test groups II and III (the drug doses of 1.130 mg/kg and 565 mg/kg, respectively) was probably due to a significant decrease in the body weight of rats during the experiment. The Test group I data (2.230 mg/kg) were biased due to the insufficient number of indices for statistical processing (two surviving rats).

The evaluation of toxic properties also included the determination of hematological and biochemical blood parameters of rats. The results of hematological studies conducted on day 15 of the experiment
(day 1 after the withdrawal of the drug) are presented in table 2.

Table 2. Hematological parameters of rat blood after 14 days of Florfenicol VS 30 injections.

| Parameter                  | Control            | Drug dosage, mg/kg       | Reference  |
|----------------------------|--------------------|--------------------------|------------|
|                            |                    | Test I (2.260 mg/kg), n=2| Test II (1.130 mg/kg), n=5 | Test III (565 mg/kg), n=5 |
| Hematocrit, %              | 44.22 ± 2.28       | 35.30 ± 4.01            | 41.68 ± 1.39 | 41.00 – 51.00 |
| Hemoglobin, g/L            | 156.60 ± 7.27      | 128.00 ± 8.49           | 146.40 ± 4.78 | 130 – 164 |
| Erythrocytes, 10^12/L      | 8.10 ± 0.45        | 6.23 ± 0.82             | 7.15 ± 0.82 | 7.63 ± 0.43 | 5.50 – 10.00 |
| Leukocytes, 10^9/L         | 4.70 ± 2.96        | 13.95 ± 4.55            | 6.20 ± 1.25 | 5.50 – 11.00 |
| Thrombocytes, 10^9/L       | 884.60 ± 85.43     | 976.50 ± 251.62         | 958.60 ± 121.79 | 300.0 – 750.0 |
|                            |                    | Stab neutrophil, %       | 0.80 ± 1.04 | 3.00 ± 1.04 | 2.80 ± 3.33 | 0.80 ± 1.04 | 1.00–4.00 |
|                            |                    | Segmented neutrophils, % | 24.80 ± 7.77 | 84.00 ± 27.61 | 51.80 ± 27.61 | 12.60 ± 9.02 | 20.00 – 35.00 |
|                            |                    | Eosinophils, %           | 1.60 ± 3.12 | 0.00 ± 2.04 | 1.20 ± 2.04 | 1.60 ± 1.88 | 1.00 – 3.00 |
|                            |                    | Monocytes, %             | 1.40 ± 1.11 | 3.00 ± 4.59 | 5.20 ± 4.42 | 5.20 ± 4.59 | 1.00 – 5.00 |
|                            |                    | Lymphocytes, %           | 71.40 ± 7.32 | 10.00 ± 26.75 | 39.00 ± 26.75 | 79.80 ± 13.38 | 55.00 – 75.00 |

*a* means statistical significance of the Control group indicator (p ≤ 0.05); *b* means that the confidence interval was not calculated due to insufficient amount of data.

According to the data presented in table 2, the morphological study of the blood of the experimental animals found a statistical significance in some Test groups indices, different from ones of Control group, i.e.

- significant decrease in hematocrit and hemoglobin in Test groups II and III, namely, 1.130 mg/kg and 565 mg/kg, respectively; and
- erythrocytopenia and thrombocytopenia at a drug dose of 1130 mg/kg.

These changes were probably caused by severe malnutrition of the laboratory animals. Statistical significance was also revealed in the leukocyte formula of rats in Test groups compared to the Control, namely,

- a decrease in the number of segmented neutrophils in Test group III (565 mg/kg); and
- an increase in the number of segmented neutrophils and monocytes, as well as a decrease in lymphocytes in Test group II (1.130 mg/kg).

The average values of the blood hematology parameters under study (hematocrit, hemoglobin, and the absolute number of formed elements) should be noted to comply with the physiological norms for this animal species [11, 12].

On day 10 after the course of intramuscular injections, the blood hematology indices of the Test rats did not differ significantly from the Control analogs.

The biochemical blood profile of Test animals during all registration periods was comparable to Control rats, though there was a decrease in the total protein in Test group II (1.130 mg/kg) on day 1 after discontinuation of the drug by 20% (53.20±6.53 g/L versus 65.80±4.42 g/L).

4. Conclusion
The conducted sub-acute experiment on outbred rats determined the toxicological properties of the medicinal product Florfenicol VS 30 (developed by OOO VETSFERA, Russia) for veterinary use. The
injected drug in the tested doses caused a significant decrease in the body weight, as well as significant changes in the hematological and biochemical blood parameters. The revealed intoxication signs at a dose of 565 mg/kg were reversible.

Summarizing the results of the experiment, we can conclude that the doses of 2.260 mg/kg and 1.130 mg/kg are toxic, and the dose of 565 mg/kg is the threshold for laboratory rats.

According to the instruction, the study drug does not pose a danger to the target animals, since Florfenicol VS 30 in clinical practice is prescribed twice with an interval of 48 hours at doses of 1.0 mL/15 kg for cattle and 1.0 mL/20 kg for pigs, which is more than 7 times lower than the threshold dose of the sub-acute experiment.

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