Toxicological evaluation of a new iron-containing preparation for farm animals with alimentary anemia

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Abstract. The second for the significance livestock sector is pig breeding, which accounts for one-third of the country's meat production. Long-term world practice of pig farming confirms the high maturity of this species of animals, excellent taste, nutritious meat and most importantly - the ability to quickly increase food production, and thereby ensure the optimal balance of the diet of the population. One of the constraining factors for the development of this industry is the development of iron deficiency alimentary anemia in pigs during the first 7 days of animal life, which affects the growth, development and increase of live weight in animals. Without preventive measures to supplement the iron with animals, up to 100% of piglets become sick with anemia, which can lead to the death of a significant part of the young population. This article presents the results of the toxicological approbation of a new iron-containing preparation for farm animals. The question of finding a less toxic iron preparation with a higher prolonging effect is relevant.

1 Introduction

Iron is one of the most important trace elements for vertebrates, which is involved in many biochemical processes [1]. A unique property of these proteins is the formation of a temporary compound with an oxygen molecule without changing the degree of oxidation of iron Fe^{2+} . Hemoglobin is found in red blood cells, transporting oxygen from the lungs to the cells, and carbon dioxide back from the cell to the lungs. This function is responsible for the regulation of acid-base balance in a living organism, being the main buffer system of the blood. Since mammals cannot synthesize iron, mechanisms to control the intake, save, and distribution of iron, both at the systemic and cellular levels have evolved [2]. Hepcidin, a peptide produced by the liver and secreted into the blood circulation, regulates iron metabolism by inhibiting its excretion from cells [3, 4].

Iron deficiency anemia (IDA) is considered the most common nutritional deficiency worldwide. Newborn piglets are an ideal model for studying the multifaceted etiology of iron deficiency anemia in mammals since iron deficiency anemia is the most common

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deficiency disorder during the early postnatal period in this species and often develops into a critical illness. Hepcidin production by hepatocytes decreases in response to stimulation of the export of iron from cells into the bloodstream with a lack of serum iron or with an increased level of erythropoiesis. At the same time, hepcidin production increases in response to the need to reduce iron export from reticuloendothelial cells to the systemic circulation with an excess of serum iron and inflammatory processes [5].

Alimentary anemia of piglets is accompanied by the development of secondary immune deficiency, which sharpens age-related immune deficiency, thereby increasing the risk of secondary diseases of the digestive and respiratory systems, and, consequently, economic losses [6]. A common cause of IDA for piglets is the contradiction between the high iron requirement due to rapid growth and insufficient endogenous supply. A hemoglobin concentration of 8 g/dl is considered the threshold level of anemia in suckling pigs [7, 8], which decreases to the level of 6–7 g/dl on the 3rd day after birth [9]. A low iron concentration in pig milk and an increased need for iron for rapid growth are usually considered as risk factors for IDA in piglets [10, 11]. To prevent the development of this disease, an exogenous source of iron should be administered orally or by injection [12, 13]. Oral administration of iron in piglets is unsuccessful due to poor digestibility in the form of less secretion of gastric acid, poor motility of the gastrointestinal tract and immature system of absorption of piglets, as well as high toxicity since this iron is a transition metal that changes its valency [13]. Parenteral (intramuscular or subcutaneous) injection of exogenous iron in order to avoid IDA in piglets was well approbated and implemented [14].

Currently, the search, development and introduction of drugs (mainly of natural origin), which in their complex will additionally contain the necessary trace elements, are becoming increasingly relevant. An important role in the treatment of iron deficiency anemia is currently given to vitamins that are part of the drugs. Their incorporation into the composition of iron-containing drugs positively affects erythropoiesis and the digestibility of iron by the body. In this case, special attention is paid to the development and testing of technologically advanced in use drugs with low toxicity, high bioavailability when used in newborn piglets.

Among the iron-containing compounds that are used in the prevention and treatment of IDA, iron-based preparations based on chelate complexes are becoming increasingly popular. They easily establish an ionic bond with the cells of the body, disintegrate and are completely absorbed. All chelate compounds have a heterocyclic ring that provides stability and optimal absorption of trace elements by the body. Chelates do not require additional transformations in the body, they are ready for use and transportation. An additional intake of chelating drugs is able to guarantee the satisfaction of the body's needs in microelements and their complete assimilation. Vitamin and mineral preparations containing such forms of trace elements are the most effective which is important in the treatment of iron deficient nutritional anemia.

2 Materials and Methods

The aim of the study was a pharmaco-toxicological evaluation of a new iron-containing drug for the prevention of nutritional anemia in piglets.

Within the framework of the study, a preparation based on a chelate complex of Fe^{3+} iron with gluconic acid, the central ion of which is an iron ion, and ligands are residues of gluconic acid was proposed. The model of the iron gluconate molecule is shown in Figure 1. The formation of this complex is due to the interaction of the Fe^{+3} iron ion with negatively charged carboxyl groups and hydroxy groups located in the α -position of three gluconic acid molecules.

Fig. 1. The model of iron (III) gluconate molecule.

Studies of pharmacological and toxicological properties were carried out at the Department of Therapy and Pharmacology in the vivarium of the Faculty of Veterinary Medicine of the Federal State

Budgetary Educational Institution of Higher Education «Stavropol State Agrarian University». The experiment involved 156 white non-linear mice and 96 white non-linear rats. In the study of a new drug, it was determined in comparison, not less than 2 types of laboratory animals. The experiments used clinically healthy animals that had not previously been exposed to toxic effects: white mice weighing about 18-25 g and white rats weighing about 150-200 g.

The maintenance and care of laboratory animals were carried out in accordance with the feeding diets for laboratory animals in accordance with Russian Standards. All the rules of the European Directive 2010/63/EC on the protection of animals used for scientific purposes have been observed [15].

When determining acute toxicity, preparations were administered to animals intragastrically, after 12-hour fasting, using disposable sterile medical syringes from 1.0 ml to 5.0 ml in volume via cut and sanded injection needle, in compliance with aseptic and antiseptic rules. When the drug was administered, the animals were fixed in an upright position with their head slightly tilted back, the solution was injected slowly. Feeding was carried out 2 hours after the administration of the drugs.

When studying the acute toxicity of the “Therapeutic and Prophylactic Chelated Iron-Containing Preparation” 100 laboratory mice were used, the mice were divided into 10 groups, where the first group was the control and 9 experimental groups. The drug was administered to animals in increasing doses, the starting dose was 1038 mg/kg body weight, it was administered in animal group No. 2. For mice of 3–10 groups, the drug was administered with a constant multiplicity in doses of 1177, 1316, 1455, 1594, 1733, 1872, 2011 and 2150 mg/kg. The control group (1) was injected with an appropriate volume of distilled water. The starting point in the search for a dose was the well-known data when studying the acute toxicity of iron-based compounds.

When studying the maximum tolerated doses for a single intragastric administration, 48 white non-linear rats were used. The animals were divided into 6 groups of 8 animals each (Table 3). The drug was administered to animals in increasing doses. The first group served as a control, they were administered with the appropriate volume of distilled water, in the group of animals No. 2 the dose of the drug was 1000 mg/kg, rats of groups 3-6 were administered the drug at doses of 1500, 2000, 2500 and 3000 mg/kg, respectively.

To assess the toxic effect of drugs in toxicology, it is customary to determine lethal doses (LD), which are the amount of a substance that causes the death of a certain number of animals, expressed as a percentage. There are LD₀ (minimally toxic dose), LD₁₆, LD₅₀

(mid-lethal dose), LD 84 and LD100 (absolutely lethal dose). In toxicological studies, when analyzing the effects of various drugs, it is necessary to calculate the effective doses in 50% of cases (LD50), as well as LD16 and LD84 and toxic doses: LD16, LD50, LD84 and LD100. In the study of the toxic effects of the drugs, the number of dead and surviving animals, the percentage of mortality and its expression in probits were taken into account. Dead animals were anatomized and pathological changes of acute drug poisoning were revealed. A complete autopsy was performed, the results were recorded in the protocol.

3 Results

3.1 The study of acute toxicity of “Therapeutic and Prophylactic Chelated Iron-Containing Preparation” on white mice

In the first 9 groups of mice, there were no changes in behavior, refusal of food and water, and deterioration of the physiological state of animals. In animals of the 10th group, which were administered the drug at a dose of 2150 mg/kg, we observed deep oppression, which lasted about 2 hours. Then the condition of the animals in the tenth group returned to normal. Since the animals of the 10th group showed the first signs of toxic poisoning by the studied “Therapeutic and Prophylactic Chelated Iron-Containing Preparation”, but we did not observe any lethal outcomes, the dose of 2150 mg/kg was taken as the maximum tolerated dose (MTD) and the starting one for determining lethal doses (LD).

When determining lethal doses, 7 groups were used, which were administered “Therapeutic and Prophylactic Chelated Iron-Containing Preparation” intragastrically, taking into account the rules of asepsis and antiseptics. The animals in the control group were administered with an appropriate volume of distilled water. According to the guidelines after poisoning the animals, their condition was monitored for 14 days.

The “Therapeutic and Prophylactic Chelated Iron-Containing Preparation” was administered with an increasing constant multiplicity, taking into account the number of dead and surviving animals, the percentage of mortality and its expression in probits (Table 1).

Table 1. Scheme of the experiment and the results of the study of acute toxicity of the “Therapeutic and Prophylactic Chelated Iron-Containing Preparation” on white mice.

Num. of group	The dose of preparation, mg/kg	Number of animals in the group at the beginning of the experiment	Number of dead animals	Number of survived animals	Mortality, %	Probits
1	2150	8	0	8	0	3.13
2	3150	8	2	6	25	4.33
3	4150	8	3	5	37.5	4.68
4	5150	8	6	2	75	5.67
5	6150	8	7	1	87.5	6.15
6	7150	8	8	0	100	6.87

For white mice, the average lethal dose was:

$$LD50 = ((5300 \cdot 25) + (7300 \cdot 12,5) + (9300 \cdot 37,5) + (11300 \cdot 12,5) + (13300 \cdot 12,5)) / 200 = 880000 / 200 = 4400 \text{ mg/kg.}$$

Based on the data, a probit chart was obtained (Figure 2). The values of LD16 and LD84 were determined graphically by the probit analysis method, according to the graph, the first value corresponds to probit 4, the second to probit 6.

Fig. 2. Probit chart for the “Therapeutic and Prophylactic Chelated Iron-Containing Preparation” for white mice.

MLD50 for acute toxicity for white mice was:

$$MLD50 = (6039,9 - 2951,1) / (32 \cdot 2) = 3088,8/64 = 48,26 \text{ mg/kg}$$

The acute toxicity parameters of the “Therapeutic and Prophylactic Chelated Iron-Containing Preparation” for white mice are presented in Table 2.

Table 2. The acute toxicity parameters of the “Therapeutic and Prophylactic Chelated Iron-Containing Preparation” for white mice.

Animal	MTD	LD ₁₆	LD ₅₀	LD ₈₄	LD ₁₀₀	MLD ₅₀
White mice	2150.0	2951.1	4400	6039.9	7150.0	48.26

LD50 for the “Therapeutic and Prophylactic Chelated Iron-Containing Preparation”, with a single intragastric administration was 4400 mg/kg, which, in accordance with GOST 12.007–76, belongs to hazard class 4, that means it has low toxicity.

3.2 The study of acute toxicity of “Therapeutic and Prophylactic Chelated Iron-Containing Preparation” on white rats

In the 6th group, signs of toxic poisoning were observed, the animals were apathetic and depressed, which affected the consumption of feed and water. Since the dose of 3000 mg/kg in the 6th group did not cause the death of the animals, it was defined as the maximum tolerated (MTD) and the starting one for determining lethal doses (LD).

To determine lethal doses of the “Therapeutic and Prophylactic Chelated Iron-Containing Preparation”, 5 experimental groups with 8 animals in each were formed. The animals were observed for 14 days after a single intragastric administration of the drug while taking into account their condition and behavior.

The “Therapeutic and Prophylactic Chelated Iron-Containing Preparation” was administered with a constant multiplicity, taking into account the number of dead and surviving animals, the percentage of mortality and its expression in probits (Table 3).

Table 3. Scheme of the experiment and the results of the study of acute toxicity of the “Therapeutic and Prophylactic Chelated Iron-Containing Preparation” on white rats

Num. of group	The dose of preparation, mg/kg	Number of animals in the group at the beginning of the experiment	Number of dead animals	Number of survived animals	Mortality, %	Probits
1	3000	8	0	8	0	3.13
2	4200	8	1	7	12.5	3.85
3	5400	8	3	5	37.5	4.68
4	6600	8	6	2	75	5.67
5	7800	8	8	0	100	6.87

For white rats, the average lethal dose was:

$$LD50 = ((7200 \cdot 12,5) + (9600 \cdot 25) + (12000 \cdot 37,5) + (14400 \cdot 25)) / 200 = 1050000 / 200 = 5250 \text{ mg/kg.}$$

Based on the data, a probit chart was obtained (Figure 3). The values of LD16 and LD84 were determined graphically by the probit analysis method, according to the graph, the first value corresponds to probit 4, the second to probit 6.

Fig. 3. Probit chart for the “Therapeutic and Prophylactic Chelated Iron-Containing Preparation” for white rats.

MLD50 for acute toxicity for white rats was:

$$MLD50 = (7110 - 4638,2) / (24 \cdot 2) = 2471,8 / 48 = 51,5 \text{ mg/kg (Table 4)}$$

Table 4. The acute toxicity parameters of the “Therapeutic and Prophylactic Chelated Iron-Containing Preparation” for white rats.

Animal	MTD	LD ₁₆	LD ₅₀	LD ₈₄	LD ₁₀₀	MLD ₅₀
White rats	3000.0	4638.2	5250.0	7110.0	7800.0	51.5

LD50 for the “Therapeutic and Prophylactic Chelated Iron-Containing Preparation”, with a single intragastric administration on white rats was 5250,0 mg/kg, which, in accordance with GOST 12.007–76, belongs to hazard class 4, that means it has low toxicity.

4 Discussion

As a result of the analysis of references, one can draw a conclusion about the relevance of the current work for veterinary science and practice as well as for clinical and therapeutic justification for the use of iron-containing preparations in pig breeding.

It is known that the main cause of nutritional anemia is iron deficiency, which occurs due to a mismatch between the growth rate of newborns and the low intake of trace elements with mother's milk, restructuring of the blood production, a large daily consumption of iron due to the rapid development of the piglet and an increase in blood volume [16].

In newborn piglets, a lack of iron causes a delay in growth and development, a weakening of resistance, and often death. Iron deficiency has a negative effect on the blood system, causing the development of anemia. In intensive pig breeding and the absence of prophylaxis, up to 100% of newborn piglets become sick with anemia, and their mortality can reach 30–35%.

The most commonly used method for the prevention of anemia is the parenteral use of iron dextran. An alternative to this method is the oral administration of iron preparations [17]. The use of oral drugs in industrial pig breeding is not common due to the inconvenience of using and controlling the dosage of the drug. Parenteral drugs are more convenient to dose in comparison with oral [18].

Therefore, the conduct of modern pig breeding requires the search for new economical, affordable and technological means that provide timely comprehensive prevention of iron deficiency anemia in piglets. The search, development, and introduction of drugs additionally containing the necessary trace elements are important and relevant tasks. A significant role in the treatment of iron deficiency anemia is currently given to vitamins as part of the drugs that positively affect erythropoiesis and iron absorption by the body.

Based on the foregoing, the aim of the study was to investigate the pharmacological and toxicological properties of a new iron-containing preparation for the pig breeding industry.

The development of new iron-containing preparations became possible due to the integrated use of theoretical and experimental research methods.

The developed theoretical statements and new technical solutions have been approbated experimentally.

The results can be used for the development of iron-containing complexes with high bioavailability and low toxicity, as well as in the development of a comprehensive evidence-based system of therapeutic and preventive measures for anemia of agricultural and small domestic animals.

5 Conclusions

The maximum tolerated dose of the “Therapeutic and Prophylactic Chelated Iron-Containing Preparation” for white mice was 2150.0 mg/kg body weight, LD50 was 4400.0 mg/kg, and LD100 was 7150.0 mg/kg. For white rats, the maximum tolerated dose was 3000.0 mg/kg, LD50 was 5250.0 mg/kg, and LD100 was 7800.0 mg/kg. The data obtained evidence that in accordance with GOST 12.007–76 the developed “Therapeutic and Prophylactic Chelated Iron-Containing Preparation” belongs to hazard class 4, i.e. low toxicity substance.

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