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Toxicodynamic Study The "Startin-Phyto" Drug.

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ABSTRACT

The article presents the results of studying the toxicodynamics of the drug “Startin-Phyto”. On the basis of the research, it was established that the studied preparation is of low toxicity and safe when used daily by animals. The study drug does not provoke the development of pathological reactions.

Keywords: Dyspepsia, toxicodynamics, toxicity, Startin-Phyto, LD50, total protein, urea, glucose, creatinine, aspartate - and alanine aminotransferase (AsAT and AlAT) and lactate dehydrogenase (LDH).

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INTRODUCTION

Dyspepsia in calves belongs to the group of neonatal diseases of the young (in the first days after birth). It is observed more often in calves and piglets. Dyspepsia is a digestive disorder in newborn calves and piglets with a sign of diarrhea (diarrhea). Dyspepsia is an acute disease of calves of the colostrum period, characterized by indigestion (diarrhea), metabolic disorders (especially water-electrolyte) and intoxication of the body. In physiologically mature offspring, indigestion develops with overfeeding, deterioration of the quality of colostrum (cold, polluted). Dysbacteriosis, toxicosis, diarrhea, dehydration, loss of appetite develop. The disease is more common in the winter-spring period. The incidence is up to 100%, the mortality rate is 20–50–80% [1].

Despite the progress achieved in the treatment and prevention of dyspepsia in newborn calves, the problem associated with the development of effective drugs for the treatment of the disease remains.

In connection with the increasing importance of medicinal plants in medicine and veterinary medicine, we decided to study the mechanism of the toxic action of the herbal medicine “Startin-Phyto”.

MATERIALS AND METHODS

To determine the indicators of acute toxicity of the drug "Startin-Phyto" in rats and mice, groups of 6 animals of the same sex were used. In addition, there were similar in number of groups of control animals of each sex. “Startin-Phyto” is a combined preparation, which is a package of powdery substances with a total weight of 558 g, packaged in four bags of plastic film. Package No. 1 (sodium carboxymethylcellulose or methylcellulose) is a white to brownish gray fine-fibrous powder. Package # 2 (glucose) - powder or granules of white or slightly yellow color. The contents of package No. 3 (sodium chloride, lactic acid calcium, ascorbic acid) are white powder. Package # 4 (Hypericum extract) is a dark-colored powder. Packages (№№ 1, 2, 3, 4) are placed in a package (outer) of polyethylene film, in which they put a label indicating the name of the preparation, the manufacturer, the series number (batch), the quantity of the preparation, the expiration date and the date of manufacture. The drug is stored in a dry, dark place at a temperature not higher than 300C. The shelf life of the drug - 1 year from the date of manufacture. "Startin-Phyto" is used by newborn calves for the prevention and treatment of acute gastrointestinal diseases. The drug is used in the form of a solution. To do this, before using the contents of three packages dissolved in 10 liters of hot water (700C), carefully rubbing floating lumps. The resulting solution is poured into glass or enamelware and left for a day at room temperature, stirring several times during the day. The Startin-Phyto solution is suitable for use within 5 days after preparation. For prophylaxis to calves, the first 6 feeds after birth for two days add 250 ml of Startin-Phyto solution to each one-time portion of colostrum and drink it from the teat drinker. With the purpose of treatment, a calf in the next two feeds instead of milk is given 250 ml of the Startin-Phyto solution with the addition of 0.5-0.7 liters of warm water. Then, to each next portion of milk, add 250 ml of the Startin-Phyto solution and warm drinking water and bring up to the total volume of the portion 1.25-1.5 l. The drug is given to the clinical recovery of the animal. Start-Phyto solution is preheated to a temperature of 37-38°C before use.

In appearance, the Startin-Phyto solution is a homogeneous dark-gray jelly-like liquid with a specific odor.

The study "Starting-Phyto" was carried out in accordance with the methodological recommendations of the Pharmacological State Committee [7, 8].

Preparation for the experience of laboratory rodents was carried out in accordance with GF XI "Test for toxicity." Before the experiment, the animals were cleaned food and water. After two hours, the animals were weighed and divided into groups [4].

To determine the indicators of acute toxicity, the drug Startin-Phyto was administered to white mice and rats intragastrically (W / W). White male mice weighing 25-30 g of "Startin-Phyto" were administered in doses of 10,000 and 25,000 mg/kg. For the introduction of each dose used 6 white mice. Animals of the control group (6 white mice) were injected with distilled water - 0.5 ml. White male rats weighing 124-170 g of “Startin-Phyto” were administered in doses of 18,000 and 21,400 mg/kg. For each dose, 6 white rats were

used. The control group (6 white rats) were injected with distilled water - 3 ml. The calculation of LD50 was performed by the method of probit analysis proposed by Litchfield-Wilcoxon in the modification of Z. Roth [2]. After oral administration of the drug, an atraumatic metal probe was used, which was immersed in the stomach. Control animals were injected with similar volumes of boiled water. To achieve large doses (more than 21,400 mg/kg), the drug was re-administered at intervals of 60 minutes 3 times. Control animals were injected with similar volumes of boiled water (solvent). Animals were observed for 14 days.

For 14 days, we monitored the general condition and behavior of animals, the manifestation or absence of symptoms of intoxication; noted the peculiarities of behavior, food and water intake, estimated the condition of coat, the departure of physiological functions, etc. A total of 12 mice and 12 rats were used in the experiment. After 14 days, groups of experimental rats were euthanized (rats) and subjected to pathological examination.

In order to characterize the general state of animals during the study of the Startin-Phytotoxicodynamics, the total protein, urea, glucose, creatinine, aspartate, and alanine aminotransferase (AsAT and AlAT) and lactate dehydrogenase (LDH) were determined. When conducting experimental studies, the determination of total protein was performed according to the biuret reaction. The principle of the method is that proteins react in an alkaline medium with copper sulfate, with the formation of compounds colored in purple [5, 6, 12].

The glucose content was determined by the unified glucose oxidase method for orthodolidine oxidation. The principle of determination is that glucose oxidase oxidizes glucose to form hydrogen peroxide, which, under the action of peroxidase, oxidizes orthotolidine to form blue chromogen. Urea was determined by a unified method by the color reaction with diacetylmonoxime. The concentration of total bilirubin was determined by the colorimetric method with 2,4-dichloroaniline, and creatinine was determined by the color reaction Yaf [5, 6, 12].

The activity of AlAT and AsAT was determined by a unified dinitrophenylhydrazine method. The principle of the method consists in transamination under the action of AsAT and AlAT, when oxaloacetic and pyruvic acids are formed. When 2,4-dinitrophenylhydrazine is added in an alkaline medium, colored pyruvic and oxaloacetic acid hydrazones are formed [5, 6, 12].

The activity of lactate dehydrogenase (LDH) in the blood serum by the reaction of 2,4-dinitrophenylhydrazine (Sevel-Tovarek method) [5].

These experimental studies were processed by the method of variation statistics. For this purpose, the application software STATISTICA [9] was used. The statistical significance of the differences was established by the value of Student's t test.

RESULTSANDDISCUSSION

As a result of the research, it was found that the introduction of the test drug in doses of 10,000, 25,000 mg/kg did not lead to the death of mice, and there were no signs of intoxication. In these groups, it was not possible to determine the deadly doses of Startin-Phyto. The general condition and behavior of mice, feed and water intake, visible physiological functions remained unchanged.

The results of the study of acute toxicity "Startin-Phyto" on white mice and rats are presented in table 1 and 2.

Table 1: Toxicity of the drug "Startin-Phyto" when i/w administered to mice

Dose mg/kg	10000	25000
Volume of inputsolution ml	0,25	0,7
Effect, fell/total	0/6	0/6

The results of the study of acute toxicity "Startin-phyto" on white rats are presented in table 2.

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Table 2: Toxicity of the drug "Startin-Phyto" with i/f administration to rats

Dose mg/kg	18000	21400
Volume of inputsolution ml	2,5	3
Effect, fell/total	0/6	0/6

None of the cases of lethal effects are registered even with the introduction of maximum doses: 21400 ml/kg for intragastric use. Also, there were no significant violations of the general condition and behavior of animals. Some thirst and a decrease in feed intake on the first day were observed in the control group of animals, and are connected, apparently, not with the toxic effect of the drug, but with stress during the oral administration of large volumes. On all days of observation on the general condition and behavior, the experimental animals did not differ from the controls.

The dynamics of body weight in all groups remained normal. Table 3 shows the data on measuring the body mass of animals in all experimental groups.

Table 3: Dynamics of body weight of rats after intragastric administration of the drug "Startin-Phyto" (P≥0.05)

Observation time	Study groups (g, M ± m)	
	Control	Startin-Phyto
Before the introduction	134,0±8,57	139,8±8,04
2nd day	133,0±8,46	136,8±8,1
5th day	134,8±8,65	139,8±8,2
14th day	138,4±8,86	145,0±7,94

From table 3 it can be seen that on the second day of the study, there was some loss of body weight in animals of all groups, including the control group, apparently caused by stress associated with the introduction of the drug and placing the animals in a research environment. Data analysis did not reveal any significant differences in the dynamics of body weight between the experimental and control animals. A slightly larger increase in body weight in animals of the experimental groups as compared to the control ones by the end of the study was not statistically significant and had only a tendency character.

The results of biochemical parameters of the blood of experimental animals 14 days after the introduction of "Startin-Phyto" are presented in table 4.

Table 4: Biochemical blood parameters of rats 14 days after enteral administration of "Startin-Phyto" at a dose of 21,400 mg/kg (P≥0.05)

Indicators	Control	Startin-Phyto
Total protein, g / l	55,1±2,27	57,14±0,98
Glucose, mmol / l	4,06±0,38	3,98±0,31
Bilirubin, mol / l	2,56±0,18	2,22±0,07
Urea, mmol / l	1,77±0,27	1,56±0,25
Creatinine, µmol / L	37±2,43	38,2±0,86
AlAT, E / l	63,8±3,07	111,8±20,4*
AsAT, E / l	283,8±38,3	293,4±34,9
LDH, E / l	1262,8±109,9	1156,6±116,98

* where is p <0,05

Analyzing the data of table 4, it should be noted that in experimental animals there is a significant increase in the activity of AlAT. When studying the amount of total protein, glucose, bilirubin, urea, creatine, the activity of the enzymes AsAT and LDH, no significant difference was found between the experimental and control groups.

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A macroscopic examination has not established the effect of the drug on the state of the internal organs. The results of studies of weight, the mass of internal organs and weights are presented in table 5.

Table 5: Mass and weight coefficients of rgans in white rats after a dose of 21.4 ml / kg of the drug "Startin-Phyto" (P≥0.05)

Indicators	Control	Startin-Phyto
Mass of rats	138,4±8,9	145,8±9,3
Liver, g	5,6±0,26	7,36±0,63*
Liver weight	40,7±1,22	52,6±6,21
Spleen, g	0,72±0,12	0,75±0,07
Spleen weight	5,2±0,58	5,3±0,69
Kidney, g	1,2±0,05	1,46±0,09*
Kidney weighting	8,6±0,35	10,3±1,1
Light, g / l	1,7±0,09	2,01±0,11
Lung weights	12,2±0,56	14,1±1,53
Heart g	0,58±0,05	0,69±0,07
Heart weighting	4,2±0,16	4,8±0,49
Seed plants, g	2,5±0,34	3,07±0,3
The weighting factor of the testes	17,7±1,85	21,5±2,93

* where is p <0,05

Analyzing the data of table 5, it should be noted that in experimental animals there is a significant increase in the mass of the liver and kidney. In all likelihood, this is due to increased anabolic processes under the influence of the drug. When studying the weight, the mass of other internal organs, weight ratios and blood biochemical parameters of experienced rats did not find any significant difference from similar indicators of rats in the control group. In the autopsy study, the effect of the drug on the state of the internal organs was not established. Wool rats had a neat appearance, was brilliant, without pockets of baldness. Skin and subcutaneous tissue unchanged. Infiltrates, irritation or tissue necrosis was not observed. When viewed from the thoracic and abdominal cavities, there were no violations in the location of the internal organs. The size and shape of the heart without changes. The muscle of the heart was brownish, dense. The surface of the lungs had a pale pink color; lungs collapsed when opening the chest. The fabric on the cut also had a uniform pale pink color. The mucous membrane of the extrapulmonary bronchi was smooth, brilliant, pale pink. The stomach had the usual shape and size, the lumen was filled with dense food contents. The mucous membrane of the body of the stomach was pale pink, shiny, folded. The mucosa of the small intestine and colon was shiny, smooth. The size and shape of the liver unchanged, the liver capsule was thin, transparent. The liver tissue had a brownish color and a moderately dense texture. The size and shape of the kidneys did not differ from the control, the capsule was easily removed. The surface of the organ was a smooth, uniform brownish-grayish tint. On the incision of the kidneys, the cortex and medulla were clearly distinguished. The shape, size, and density of the testicles did not differ control. The spleen had a dark cherry color, a smooth surface, and a dense consistency. The mass coefficients of the internal organs of the animals treated with the preparation, as well as of the control animals, are shown in Table 5. There was no statistically significant change in the relative weight of any internal organs.

Thus, 2 weeks after the administration of “Startin-Phyto”, the indicators of animals from the experimental group did not differ from those of animals of the control group.

The LD_{50} values of the drug for i / f administration for outbred white rats cannot be determined and are obviously higher than 15,000 mg/kg. The preparation “Startin-Phyto” with its intravenous injection in doses up to more than 21.4 ml / kg (which is more than 21000 mg / kg) to purebred white rats did not lead to the death of animals, did not cause changes in the biochemical parameters of blood, macroscopic changes in the internal organs, did not cause swelling of the internal organs, as evidenced by the values and their mass coefficients. With a w / w injection, irritation of the mucous organs of the gastrointestinal tract was noted.

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CONCLUSION

Experimental studies have shown that the drug "Startin-Phyto," when administered to rats, has almost no toxic effect. This is evidenced by experimental data.

The levels of lethal doses of the drug "Startin-Phyto" for outbred white rats with i / f administration are obviously higher than 15,000 mg/kg. The drug “Startin-Phyto” when administered to the stomach in the maximum possible (for natural reasons) doses of white rats did not lead to the death of animals, did not cause macroscopic changes in the internal organs of animals, edema of internal organs, which is confirmed by the values of their mass coefficients.

In acute experiments on the use of the drug "Startin-Phyto," there were no signs indicating an irritant effect of the drug at the injection site.

Thus, the results of toxicometry, observational data for experimental animals in the period after administration, as well as the data of pathomorphological studies allow us to attribute the drug Startin-Phyto to the 6th class of practically non-toxic medicinal substances (table 6) [11]. The condition of animals that survived acute experiments indicates a good tolerability and harmlessness of the drug. Studies have shown that there are no contraindications in terms of toxicodynamics and acute toxicity for clinical trials or for veterinary use of the drug Startin-Phyto.

Table 6: Toxicity Grades (by Hodge and Sterner) [11]

Degree of toxicity	Term	LD_{50}, once per os, rats (mg / kg)
1	Extremely toxic	<1
2	Highly toxic	1-50
3	Moderately toxic	50-500
4	Low toxicity	500-5000
5	Practically non-toxic	5000-15000
6	Relatively harmless	>15000

According to GOST 12.1.007-76 LD50 of the drug is more than 5000 mg / kg, which can be attributed to the 4th class of hazard - low-hazard substances.

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