РОЗДІЛ 1. Здоров'я та благополуччя тварин
SECTION 1. Health and welfare of animals

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ACUTE TOXICITY OF THE VETERINARY MEDICINAL PRODUCT BASED ON MELOXICAM

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Abstract*. Laboratory studies were conducted to determine the acute and subacute toxicity of the veterinary drug "Loksidev" (solution for injections) on white rats, white mice, rabbits and dogs.*

The drug "Loksidev" (solution for injections) belongs to non-steroidal anti-inflammatory drugs and is used to treat animals with diseases: European fallow deer, deer - inflammatory processes of the musculoskeletal system (traumatic edema, arthritis, arthrosis, bursitis, tendinitis, etc.), acute respiratory infections, purulent catarrhal mastitis (in combination with antibiotic therapy); sports horses - acute and chronic inflammatory diseases of the musculoskeletal system (traumatic edema, arthritis, arthrosis, bursitis, tendinitis, tendovaginitis, laminitis, pododermatitis, purulent-inflammatory hoof lesions (in combination with antibacterial agents), colic.

According to the results of determining the parameters of the acute toxicity of the drug "Loksidev" (solution for injections) in the case of a single intragastric administration, the LD_{50} for female rats (based on the absolute weight of the drug) is 6375,93±494,70 mg/kg of body weight, which allows in terms of toxicity, it can be classified as class V - practically non-toxic substances (LD_{50} 5001-15000 mg/kg of body weight), and according to the degree of danger, it can be classified as class IV – low-hazard substances (LD_{50} >5000 mg/kg of body weight).

According to the results of determining the parameters of the acute toxicity of the drug "Loksidev" (solution for injections) in the case of a single subcutaneous injection, the LD_{50} for female rats (based on the absolute weight of the drug) is 4702,52±469,16 mg/kg, and for male mice 4849,01±531,24 mg/kg of body weight, which makes it possible to assign it to the VI class - relatively non-harmful substances ($LD_{50Subcut}$ >4500 mg/kg of body weight).

Key words: "Loksidev", rats, acute toxicity, dose, lethality, toxicity.

Introduction. Effective fight against infectious diseases of various etiologies of animals in Ukraine, in particular during antibiotic therapy and in the treatment of diarrhea, purulent-catarrhal mastitis, diseases of the respiratory organs and musculoskeletal system, as well as to reduce pain in the postoperative period, it is possible, if available, to use highly effective and available concomitant anti-inflammatory and analgesic drugs.

Today, the development of non-steroidal anti-inflammatory and anti-rheumatic agents, which are an integral part of systemic therapy and have significant effectiveness and environmental safety, is still relevant. Thus, "DEVIE" LLC was offered a new drug -

"Loksidev" (solution for injections). One milliliter of the drug contains the active substance: meloxicam – 20 mg, and excipients: glycine, sodium hydroxide, meglumine, water for injections – up to one milliliter.

Meloxicam is a nonsteroidal anti-inflammatory drug of the oxicam class with analgesic and antipyretic properties. The mechanism of action is based on reducing the biosynthesis of prostaglandins, which are mediators of inflammation, as a result of inhibition of the enzymatic activity of COX-2. Partially affects COX-1, which reduces the risk of side effects.

It has pronounced chondroprotective properties and, with long-term use, does not suppress bone metabolism, so it can be used to treat diseases of the musculoskeletal system. In addition, it does not affect the course of the gestational process in cows.

After subcutaneous, intramuscular or intravenous administration, it is completely absorbed, the relative bioavailability is 89-99%. The maximum concentration in the blood is reached after 1-5 hours. Has a high degree of binding to plasma proteins, mainly to albumin (99%). When it enters the synovial fluid, the concentration of the drug is reduced by half, compared to blood plasma.

It is almost completely metabolized by enzymes in the liver and excreted with urine and feces, partially with milk. The half-life is about 20 hours. The total plasma clearance is on average 8 ml/minute (Rainsford et al., 1999; Sadowski T., 2001; Chu et al., 2008).

The drug "Loksidev" (solution for injections) is used to treat animals with diseases: European fallow deer, deer - inflammatory processes of the musculoskeletal system (traumatic edema, arthritis, arthrosis, bursitis, tendinitis, etc.), acute respiratory infections, purulent-catarrhal mastitis (in combination with antibiotic therapy); sports horses – acute and chronic inflammatory diseases of the musculoskeletal system (traumatic edema, arthritis, arthrosis, bursitis, tendinitis, tendovaginitis, laminitis, pododermatitis, purulent-inflammatory hoof lesions (in combination with antibacterial agents), colic.

The purpose of this study was to conduct a toxicological evaluation of the veterinary medicinal product "Loksidev" (solution for injections) by determining its acute toxicity on laboratory animals.

Materials and methods. The experiment was conducted on 52 female non-linear white rats (3-4) months old and weighing (210-220) g, kept under optimal vivarium conditions (Zapadniuk, 1983; Kotsiumbas et al., 2006; Karkyshchenko and Hrachev, 2010): the temperature in the room was (18±2)°C, relative air humidity (60 – 70) %, the day-night lighting cycle during the experiment was (10 – 14) hours, and the air volume in the vivarium room was changed 10 times per hour.

Rats were fed complete rodent feed. Animals had free access to water and feed.

Each animal was weighed before the start of the research. The administered doses were calculated individually according to the weight of each rat, while the volume of the drug administered intragastrically at one time did not exceed 2,5 cm^3. Determination of the range of doses for the main experiment was carried out in a previous experiment.

For this purpose, a control and three experimental groups of 4 animals each (n=4) were formed in the previous experiment based on the principle of analogues. The drug was administered in doses of 4000.0; 6000.0; 8000.0 mg/kg of body weight, based on the absolute weight of the drug, once orally using an esophageal-gastric probe. Animals of the control group were injected with PEG-400.

After taking into account the results of the previous experiment, 5 experimental groups were formed in the main experiment, rats were administered the drug in doses of 4000.0; 5000.0; 6000.0; 7000.0 and 8000.0 mg/kg of body weight, as well as a control group, which animals were injected with PEG-400 in a volume of 2,0 cm^3 according to a similar protocol. There were 6 animals in each group (n=6).

It should be noted that the manipulations on rats were carried out in accordance with the existing regulatory documents, which regulate the organization of work with the

use of experimental animals and compliance with the principles of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986).

According to the clinical condition, experimental animals were observed for 14 days, noting the appearance and development of clinical signs of poisoning, the time of death or recovery to the physiological norm. During the clinical examination of rats, attention was paid to behavior, reaction to external stimuli, presence of appetite, skin condition, color of mucous membranes, frequency of breathing and defecation, changes in color and consistency of feces, etc (Zapadniuk, 1983; Kotsiumbas et al., 2006; Karkyshchenko and Hrachev, 2010).

After the death of the animals, a pathological autopsy was performed. The macroscopic method of research was used to establish patho-anatomical changes (Zharov et al., 2003). Pathological autopsy was performed according to the following scheme:

- at the first stage, an external examination was performed, noting the condition of the fur coat and mucous membranes;

- on the second - an autopsy and examination of the body cavities and internal organs, such as the pharynx, trachea, larynx, heart, lungs, liver, spleen, kidneys, stomach, intestines, was performed, noting changes in the color, consistency, pattern and shape of the organs.

Based on the results of death, the LD_{10}, LD_{16}, LD_{50}, LD_{84}, LD_{90}, LD_{100} and the LD_{50} error were calculated using the probit analysis method as modified by V. B. Prozorovsky.

The obtained results were processed by methods of variational statistics using the StatPlus 7.6.5.0 software package. Data were presented as mean values with standard deviation at the 95% confidence level.

Results. In a previous experiment, the drug "Loksidev" (solution for injections) was administered to rats in doses of 4000.0; 6000.0; 8000.0 mg/kg of body weight. Clinical observations showed that intragastric administration of the drug to rats of experimental group I (4000.0 mg/kg of body weight), 20-30 minutes after administration, caused slight depression, which increased during the first day after administration, the animals reluctantly accepted feed and water, slowly moved around the cage. Reaction to external stimuli was reduced, impaired coordination of movements was observed. On the first day and for 4 days after administration, no movement coordination disorders were observed in the animals, however depression, decrease in feed consumption and thirst were noted, in two animals from the group feces were of a liquid consistency. From the 4th to the 8th day, only a reduced feed intake and a dull shade of the fur coat were observed in the rats. Deaths of rats in this group were also not observed during the 14-day observation period, however, their clinical condition was restored only by the end of the experiment (Table 1).

In the rats of the II experimental group (6000.0 mg/kg of body weight), during the first day after the administration, more and more depression was noted, the animals refused food and water. Further, a violation of coordination of movements was observed, the animals reluctantly moved around the cage, mostly sat in one place. Within 2-3 days after the introduction, there was no violation of coordination of movements, however, at this time, the animal had a small consumption of feed and a liquid consistency of feces. A similar condition was observed in rats from the 3rd to the 8th day of the experiment, while progressive exhaustion, growing depression and complete refusal of food and water were added to the clinical picture. The death of rats was observed on the 7-8th day after administration (table 1). It should be noted that two rats from the II experimental group survived, their clinical condition did not fully recover until the control on the 14th day of the experiment, however, the rats resumed feed and water consumption, which served as a positive prognostic aspect.

Table 1

The dynamics of the death of rats in the previous experiment, to determine the acute toxicity of the drug "Loksidev" (solution for injections) (n=16)

Terms of death rats, through	Groups of rats and doses, mg/kg of body weight			
	Control	Experiment		
		I (4000,0)	II (6000,0)	III (8000,0)
2-3 days	–	–	**–**	–
4-5 days	–	–	**–**	**2**
6-10 days	–	–	**2**	**2**
11 - 14 days	–	–	**–**	–
All died	–	–	**2**	**4**

In the rats of the III experimental group, which were administered the drug at a dose of 8000.0 mg/kg of body weight, during the first day after administration, increasing depression was noted, the animals refused food and water, then a violation of movement coordination was observed, the animals moved reluctantly around the cage, in mostly sat in one place. Within 2-3 days after the introduction of the drug, there was no movement coordination disorder, however, at this time, the animal had low feed consumption and a liquid consistency of feces. A similar condition was observed in rats from the 3rd to the 8th day of the experiment, while progressive exhaustion, growing depression and complete refusal of feed and water were added to the clinical picture. The death of all rats from the group was observed on the 5-7th day after administration (table 1).

In the main experiment, the drug Loxidev (for injections) was administered to rats in doses of 4000.0; 5000.0; 6000.0; 7000.0 and 8000.0 mg/kg body weight. During the observation of the animals of the 1st experimental group 20-30 minutes after the administration of the drug, a slight inhibition was recorded, which increased during the first day after the administration. The animals were reluctant to accept food and water, moved slowly around the cage, the reaction to external stimuli was reduced, impaired coordination of movements was observed. On the first day after and for 4 days after administration, no movement coordination disorders were observed in the animals, however depression, decrease in feed consumption and thirst were noted. In two animals from the group, feces had a liquid consistency. From the 4th to the 8th day, only a reduced feed intake and a dull shade of the fur coat were observed in the rats. The deaths of rats in these groups were also not observed during the 14-day observation period, but their clinical condition was restored only by the end of the experiment.

A similar clinical picture of poisoning was observed in rats of the II research group (5000.0 mg/kg of body weight), however, one rat died on the 8th day. The clinical condition of the surviving rats recovered only by the end of the experiment (table 2).

Table 2

The dynamics of the death of rats in the main study, to determine the acute toxicity of the drug "Loksidev" (solution for injections) (n=36)

Groups of rats and doses, mg/kg body weight		Periods of death of rats, through				
		1-3 days	4-6 days	7-9 days	10-14 days	All died
Control		–	–	–	–	–
Experiment	I (4000,0)	–	–	–	–	**–**
	II (5000,0)	**–**	–	**1**	–	**1**
	III (6000,0)	**–**	–	**3**	–	**3**
	IV (7000,0)	**–**	**2**	**1**	–	**3**
	V (8000,0)	**–**	**4**	**2**	–	**6**

In the rats of III and IV experimental groups (6000,0 and 7000,0 mg/kg of body weight), during the first day after administration, more and more depression was noted. The animals refused feed and water, then observed a violation of coordination of movements, the animals reluctantly moved around the cage, mostly sat in one place. Within 2-3 days after the introduction, there was no impaired coordination of movements. However, at this time, the animals noted a small consumption of feed and a liquid consistency of feces. A similar condition was observed in rats from the 3rd to the 8th day of the experiment, while progressive exhaustion, growing depression and complete refusal of food and water were added to the clinical picture. The death of rats was observed on the 7th-8th day after administration (Table 2). It should be noted that 3 rats from both groups survived, their clinical condition did not fully recover until the control on the 14th day of the experiment, however, the animals resumed feed and water consumption, which served as a positive prognostic aspect.

Rats of experimental group V, to which the drug was administered at a dose of 8000.0 mg/kg of body weight, during the first day after administration, increased depression was noted, the animals refused food and water. Further, a violation of coordination of movements was observed, the animals reluctantly moved around the cage, mostly sat in one place. Within 2-3 days after the administration of the drug, there was no movement coordination disorder, however, at this time, the animal had low feed consumption and a liquid consistency of feces. A similar condition was observed in rats from the 3rd to the 8th day of the experiment, while progressive exhaustion, growing depression and complete refusal of food and water were added to the clinical picture. The death of all rats from the group was observed on the 5-7th day after administration (Table 2).

After the death of the rats, a pathological autopsy was performed. During the external inspection of the corpses of experimental animals, it was established that the corpses were exhausted, the fur was disheveled, dull; there were no discharges from the oral and nasal cavities, while the anus area was contaminated with feces; paleness of visible mucous membranes was noted.

At autopsy in rats:

- pallor of the mucous membranes of the oral cavity, trachea, pharynx and esophagus was recorded;
- the stomach is empty in some with a small amount of feed, collapsed or distended, without signs of inflammation;
- the heart is enlarged in volume, the atria are filled with blood;
- the blood is coagulated;
- the liver is enlarged in volume, from light brown to clay-colored, with a very flabby consistency;
- lungs, spleen and pancreas – unchanged; the bladder is full of urine, the kidneys are light brown, not enlarged;
- swelling and catarrhal inflammation of the mucous membrane was established in the small and large intestines.

The next stage of studying the toxicological characteristics of the Loxidev drug (for injections) was the determination of the average lethal dose and its standard error (LD_{50}, LD_{10}, LD_{16}, LD_{84}, LD_{90}, LD_{100}).

The average lethal dose LD50 was calculated using the method of probit analysis according to V. B. Prozorovsky. The toxicometric parameters of the drug were calculated using the method of least squares for probit analysis of lethality curves. The percentage of lethality, probits (Y), weighting coefficients of probits (Z) are established.

To construct the graph, the values of drug doses (mg/kg) were plotted on the abscissa axis, and the effect values (%) were plotted on the ordinate axis.

A graphic representation of the curve showing the dose-effect relationship for rats is presented in Fig. 1.

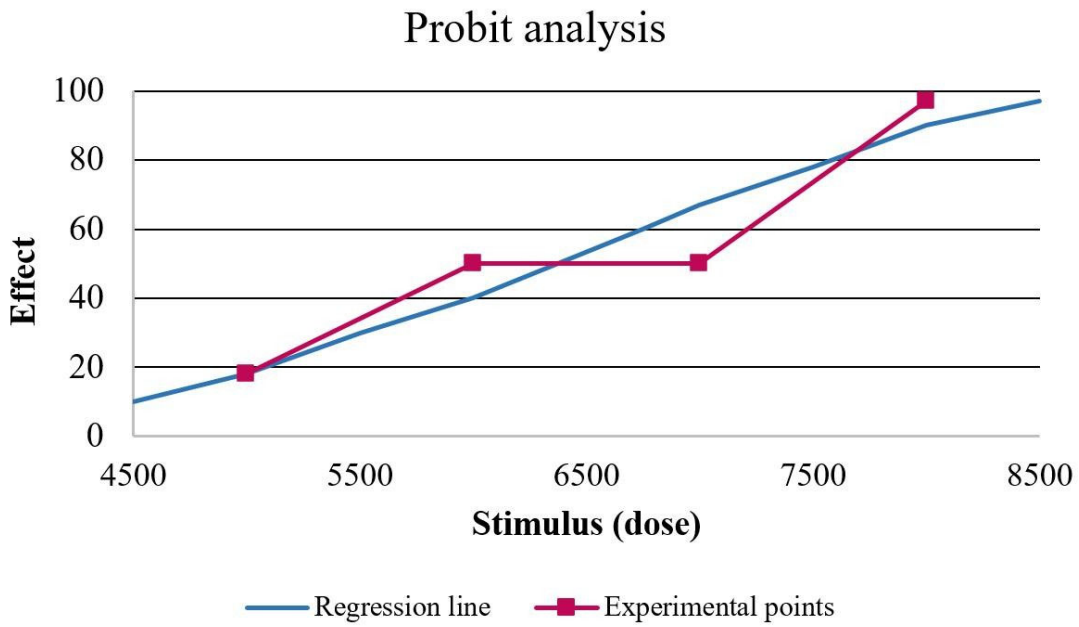

Fig. 1. The lethality curve of female rats under the conditions of a single oral administration of the drug "Loksidev" (solution for injections)

The results of calculating the average lethal dose of the drug for rats, under the conditions of oral administration, are presented in table. 3.

Table 3

The results of calculating the lethal doses of the drug "Loksidev" (solution for injections), under the conditions of a single oral administration to female rats

Stimulus (Dose)	Percent (%)	N	Probit (Y)	Weighting coefficient (Z)
4000	4,1667	6	3,2680	1,5359
5000	16,667	6	4,033	3,565
6000	50,000	6	5,000	5,000
7000	50,000	6	5,000	5,000
8000	95,833	6	6,732	1,536
Regression statistics				
LD_{50}	**6375,93**	**LD_{50} Standard error**		**494,70**
LD_{10}	4473,71	*LD_{16}*	4891,83	
LD_{84}	7860,03	*LD_{90}*	8278,15	
LD_{100}	8602,09			

Based on the results of research, it was established that the LD_{50} of the Loxidev drug (for injections), under the conditions of its single oral administration to female rats, is 6375,93±494,70 mg/kg, LD_{10} – 4473,71 mg/kg, LD_{16} – 4891,83 mg/kg, LD_{84} – 7860,03 mg/kg, LD_{90} – 8278,15 mg/kg, LD_{100} – 8602,09 mg/kg body weight, respectively.

Therefore, the drug "Loksidev" (solution for injections) can be classified in terms of toxicity to class V - practically non-toxic substances (LD_{50} 5001-15000 mg/kg of body weight), and according to the degree of danger - to class IV – low-hazard substances (LD_{50} >5000 mg/kg of body weight) (Kotsyumbas I.Ya., 2005).

Conclusions. According to the results of determining the parameters of the acute toxicity of the drug "Loksidev" (solution for injections), in the case of a single intragastric administration, the drug can be classified as toxic in class V - practically non-toxic substances (LD_{50} 5001-15000 mg/kg of body weight). and according to the degree of danger - up to IV class - low-hazard substances (LD_{50} >5000 mg/kg of body weight).

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ГОСТРА ТОКСИЧНІСТЬ ВЕТЕРИНАРНОГО ЛІКАРСЬКОГО ПРЕПАРАТУ НА ОСНОВІ МЕЛОКСИКАМУ

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***Резюме**. Проведені лабораторні дослідження з визначення гострої та підгострої токсичності ветеринарного препарату "Локсидев" (розчин для ін'єкцій) на білих щурах, білих мишах, кролях та собаках.*

Препарат "Локсидев" (розчин для ін'єкцій) належить до нестероїдних протизапальних лікарських засобів і застосовується для лікування тварин при захворюваннях: лань європейська, олені – запальні процеси опорно-рухового апарату (травматичні набряки, артрити, артрози, бурсити, тендініти тощо), гострі респіраторні інфекції, гнійно-катаральні маститити (у комплексі з терапією антибіотиками); спортивні коні – гострі і хронічні запальні захворювання опорно-рухового апарату (травматичні набряки, артрити, артрози, бурсити, тендініти, тендовагініти, ламініти, пододерматити, ураження копит гнійно-запального характеру (у комплексі з антибактеріальними засобами), коліки.

За результатами визначення параметрів гострої токсичності препарату "Локсидев" (розчин для ін'єкцій) у разі одноразового внутрішньошлункового введення LD_{50} для щурів-самок (за абсолютною масою препарату) складає 6375,93±494,70 мг/кг маси тіла, що дозволяє за токсичністю віднести його до V класу – практично не токсичних речовин (LD_{50} 5001-15000 мг/кг маси тіла), а за ступенем небезпечності до IV класу – малонебезпечних речовин (LD_{50} >5000 мг/кг маси тіла).

За результатами визначення параметрів гострої токсичності препарату "Локсидев" (розчин для ін'єкцій) у разі одноразового підшкірного введення LD_{50} для щурів-самок (за абсолютною масою препарату) складає 4702,52±469,16 мг/кг, а для мишей-самців 4849,01±531,24 мг/кг маси тіла, що дозволяє віднести його до VI класу – відносно не шкідливих речовин ($LD_{50Subcut}$ >4500 мг/кг маси тіла).

Ключові слова: "Локсидев", щури, гостра токсичність, доза, летальність, токсичність.

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