Toxicological characteristics of a combined pyrethroid preparation

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Abstract. The article presents the toxicological characteristics of the combined drug Neostomosan, intended for the possibility of combating ectoparasites of farm animals in livestock buildings. It was found that the studied pyrethroid drug belongs to low-hazard agents. When administered intragastrically to laboratory mice, LD_{50} of Neostomosan was 11,500 mg/kg. When applied locally to the skin of animals, LD_{50} of the drug exceeded 10,000 mg/kg. The cumulation coefficient of Neostomosan was 10. A single treatment of calves with 0.3% of the drug did not cause toxicosis. After application of Neostomosan in this concentration, a reliable decrease in glucose levels on the 1st and 30th days, an increase in alanine aminotransferase activity on the 5th and 15th days, and a decrease in cholinesterase activity on the 15th day were noted in the blood of calves. The studied preparation did not cause any changes in the internal organs of the calves.

1 Introduction

In modern animal husbandry, pyrethroid preparations are widely used to combat ectoparasites of farm animals [1. 2]. The range of pyrethroid preparations for animals is constantly expanding [3]

The mechanism of action of pyrethroids is to interact with sodium channels and induce prolonged depolarization in neurons [4]. Despite the fact that pyrethroids have selective insecticidal activity, it is impossible not to take into account that they are neurotropic drugs and have a pronounced neurotoxic effect [5. 6].

The veterinary pharmaceutical company Seva Sante Animale (France) has developed a combined pyrethroid drug Neostomasan. The drug is a yellowish solution with a specific odor. The synthetic pyrethroids transmix and tetramethrin included in Neostomosan are capable of acting as neurotoxins on the nervous system of ectoparasites of farm animals.

The use of veterinary drugs in clinical practice requires their comprehensive establishment by toxicological properties: it is necessary to substantiate safe levels of

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impact on the animal organism, mechanisms of general toxic, cumulative and organ-specific action [7. 8].

The aim of this work was to substantiate the safety of using the combined pyrethroid drug Neostomosan. To achieve the stated goal, the acute toxicity of this drug for laboratory mice was determined, the cumulative properties and dynamics of biochemical parameters of the blood of calves were studied after its cutaneous application.

2 Materials and methods

In the experiments, Neostomasan 55 g/l (tetramethrin 50 g/l + cypermethrin 5 g/l) produced by Seva Sante Animale (France) was used.

The studies were conducted on white mice. Neostomosan was administered to mice intragastrically and cutaneously. When determining LD_{50} [9] for intragastric administration, the test doses were selected so that each subsequent dose differed from the previous one by 1.5 times. The lowest dose causing a lethal outcome was taken as LD_{50}. To obtain a more accurate result, another dose was tested, which was intermediate between the lowest lethal dose and the maximum tolerated dose. When establishing LD50 for laboratory animals, doses of 3410, 5110, 7700 and 11500 mg/kg (0.0341, 0.0511, 0.077; 0.096 and 0.115 ml per 10 g of body weight) were tested. For each dose, 3 mongrel males weighing 18–21 g were taken. The control group of animals was given drinking water in the same volume and according to the same scheme as the test drug [10].

To study the parameters of acute skin toxicity of Neostomosan, two experimental and one control groups (5 animals in each) of white mongrel male mice weighing 24-27 g were formed. Before administering the drug to the animals, their fur was cut off in an area of 1 x 1 cm in the back area. The test drug was administered to the mice once in doses of 5000 and 10000 mg/kg (or 0.05 and 0.1 ml per 10 g of animal weight). After which each animal was placed in an individual cage for 20 minutes for complete absorption of the drug and to prevent it from being licked by other animals. Mice in the control group were administered a physiological solution. For 14 days, the clinical condition of the animals, their body weight, signs of local skin reaction, possible death, and manifestation of intoxication symptoms were observed.

The cumulative properties of Neostomosan were studied using the method of S.N. Cherkinsky and co-authors [11] on 10 outbred mice weighing 22-24 g, which provides a standardized procedure for establishing the cumulation coefficient (Kcum) with daily administration of a dose of 1/5 LD_{50}.

To study the effect of Neostomasan on biochemical blood parameters, 2 groups of black-and-white calves aged 3-4 months were collected. The experimental calves of the 1st group (5 heads) were sprayed with Neostomasan 0.3% concentration at the rate of 1 liter per animal. The calves of the second group (6 heads) were the control, which were sprayed with tap water.

Experimental calves (1st group, 5 heads) were sprayed with Neostomazan 0.3% concentration at the rate of 1 liter per animal, calves of the control group (2nd group, 5 heads) - with tap water. Before the study and on the 1st, 5th, 15th and 30th days of the experiment, blood was taken from animals in the experimental and control groups to establish its biochemical parameters according to [12].

The procedures with animals were carried out according to the standards and requirements specified in [13-15].

The data from the experimental studies were processed using the methods that we described earlier in our work [16].

3 Results and discussion

Toxicological studies of a veterinary drug are necessary for the possibility of its use in animal husbandry [17. 18].

A study of the acute toxicity of Neostomosan showed that a dose of 11,500 mg/kg is the minimum dose capable of causing a lethal outcome in experimental mice. No deaths were observed when using an intermediate dose of the pyrethroid drug, namely 10,550 mg/kg. Cutaneous application of Neostomosan to mice at doses of 5,000 and 10,000 mg/kg (or 0.05 and 0.1 ml per 10 g of animal weight) did not have a negative effect on the general condition of the animals After application, the animals showed signs of excitement, which passed after 15-20 min. The results of the study of the acute toxicity of Neostomosan are presented in Table 1.

Table 1. Acute toxicity indices of Neostomosan in laboratory mice.

Approach of use	LD_{50}, mg/kg	Therapeutic dose, mg/kg	LD_{50}/therapeutic dose
intragastric	11500	30	-
cutaneous	≥10000	30	≥333,3

During the experimental period (14 days), no signs of intoxication or death of animals were observed. No death of experimental mice was observed with local application of doses of 5000 and 10000 mg/kg; LD_{50} of Neostomosan with local application exceeds a dose of 10000 mg/kg.

During the experiment to study the cumulative properties of Neostomosan, the survival rate of the experimental mice was 80%, Kcum was equal to 10. When mice were administered the studied pyrethroid preparation at a dose of 2300 mg/kg of body weight, no significant changes in their general condition or behavior were observed. At the end of the experiment, on the 18th-20th day of administration, the rodents showed depression, rapid breathing, shortness of breath, and thirst. The results of the studies of the cumulative properties of Neostomosan are presented in Table 2.

Table 2. Cumulative properties of Neostomosan.

Approach of use	LD_{50}, mg/kg	Total dose Dk, mg/kg	Kcum
intragastric	11500	460000	10

The data we obtained allow us to classify Neostomosan into a group of drugs that do not have cumulative properties.

To treat one animal with an acaricide from ectoparasites, an average of 27-30 ml/kg of acaricide solution is required. Neostomosan is used in 0.1% concentration. An assessment of the cumulative properties of the drug under study made it possible to classify it according to the degree of danger of its toxic effect (Table 3).

Table 3. Classification of the degree of danger of toxic action of Neostomosan.

Criterion of danger	Degree of danger		
	high	moderately	few
Cumulative coefficient	1…5	> 5…10	> 10
Neostomosan			10
Dose level of toxic effect	1 ED_{50}	> 5 ED_{50}	> 10 ED_{50}
Neostomosan			+
Index of breadth of therapeutic action (LD50/therapeutic dose)	5…15	> 15…45	> 45
Neostomosan			+

According to the classification of the degree of danger of the toxic effect of drugs and veterinary drugs by the cumulation coefficient, the dose level of toxic action and the index of the breadth of therapeutic action, Neostomosan belongs to low-hazard drugs [19].

A correct decision on the impact of an individual veterinary drug on the body of animals and the degree of its danger is possible only on the basis of comprehensive toxicological studies, including biochemical analysis [20]. The latter allow us to understand deeper, hidden changes in individual systems and organs of animals [21]. Without these studies, it is difficult to judge to what extent the drug can be widely used in practice.

In recent years, much attention in veterinary medicine has been paid to the study of biochemical parameters of blood, the activity of such cellular enzymes as AlAT, AsAT and ChE [22. 23].

Spraying calves with 0.3% Neostomosan did not lead to changes in their general condition and behavior; no deaths were observed in the experimental groups. Table 4 shows the biochemical parameters of blood of young cattle after the use of Neostomosan in the above concentration.

Table 4. Biochemical parameters of calves' blood after application of 0.3% Neostomosan.

Indicators	Unit	After the introduction			
		1 day	5 days	15 days	30 days
Total protein	g/l	67,7±1,33 66,3±0,67	69,3±2,03 66,3±0,67	66,7±0,67 67,0±1,16	68,3±1,76 71,0±2,00
AsAT	U/l	25,2±1,0 21,8±1,8	25,6±1,4 24,6±0,6	26,8±2,6 20,4±4,7	27,0±3,0 28,4±1,9
AlAT	U/l	22,0±1,2 19,8±2,4	26,0±1,0* 21,4±0,8	30,4±1,9* 22,4±3,9	29,2±1,4 25,4±2,4
Glucose	mmol/l	2,03±0,13* 2,87±0,32	2,70±0,10* 2,10±0,10	1,87±0,09 2,13±0,12	1,93±0,30* 3,47±0,23
Total bilirubin	µmol/l	7,37±0,57 9,63±1,13	7,37±0,53 9,07±0,57	8,50±0,98 6,70±0,10	7,93±0,55 7,37±0,52
Urea	mmol/l	2,51±0,38 2,40±0,21	5,37±0,75 4,40±0,87	4,33±1,23 3,00±0,15	3,30±0,10 3,93±0,30
Cholinesterase	U/l	235,2±26,7 210,4±12,9	159,2±12,2 216,8±35,1	163,0±13,0* 219,2±14,8	219,2±14,8 195,2±21,0
Thymol test	U	0,33±0,06 0,33±0,06	0,35±0,05 0,28±0,02	0,27±0,02 0,27±0,02	0,27±0,02 0,38±0,11

Note: The numerator shows the results of the experimental group, the denominator - control group;

* - significant difference between experimental and control groups.

After using 0.3% Neostomosan (which exceeds the therapeutic concentration by 3 times), a reliable increase in AlAT activity was observed at all study periods. On the 5th and 15th days of the study, the increase in the activity of this indicator was significantly higher when compared with similar indicators in the control group, by 21.5% and 35.7%, respectively. The amount of glucose significantly decreased on the 1st and 30th days by 29.3% and 44.4%, respectively.

The activity of ChE was unreliably high on the 1st and 30th days after spraying by 11.8 and 12.3%, respectively, and on the 5th day after spraying, the activity of ChE unreliably decreased by 26.6%, and on the 15th day after spraying with Neostomosan, the activity of ChE significantly decreased by 25.7%. Determination of ChE activity in blood serum is of

the greatest clinical interest for the diagnosis of insecticide poisoning [24. 25]. Insecticide poisoning is accompanied by a marked decrease in ChE activity. The most sharp decrease in ChE activity occurs in severe chronic liver diseases, especially in cirrhosis [26]. In the initial stages of mechanical jaundice, a decrease in ChE activity is very rare. A clear manifestation of a decrease in the protein-synthetic function of the liver is a sharp decrease in ChE activity, while the degree of decrease in ChE activity is inversely proportional to the severity of the disease.

The amount of bilirubin on the 1st and 5th day after spraying with Neosomosan decreased by 23.4% and 18.6%, respectively, compared to the same indicator in the control. On the 30th day, the indicated indicator was unreliably higher by 7.6% compared to the control. The amount of urea on the 15th day after spraying calves with the test preparation increased unreliably by 43.3% compared to the control.

In clinical practice, in most cases, the activity level of various aminotransferases in the blood is determined in order to obtain information about the localization of damage and organ dysfunction [27].

An increase in the activity of AsAT, AlAT, thymol test and the amount of glucose indicates that under the influence of the pyrethroid drug Neostomosan at a concentration of 0.3%, a change in the functioning of the biomembranes of liver cells occurs. This leads to the release of cellular enzymes (AlAT, AsAT) from hepatocytes. Increased aminotransferase activity does not reveal the depth of liver dysfunction [28, 29].

Based on research results, we can make a reasonable conclusion that Neostomosan does not provoke the development of pathological reactions, does not have a toxic effect when used, and is safe.

Experimental studies have shown that Neostomosan has virtually no toxic effect when sprayed on calves. This is evidenced by experimental data.

When applied to the skin at the maximum concentration (0.3%) in calves, the drug did not lead to the death of animals, did not cause changes in the internal organs of animals and biochemical parameters of the blood of calves.

Toxicological and biochemical blood tests show that the pyrethroid drug Neostomosan does not have a toxic effect when used and can be classified as a safe drug.

4 Conclusions

Thus, the combined drug Neostomosan, intended for the possibility of combating ectoparasites of farm animals, is classified as a low-hazard drug. When administered intragastrically to laboratory mice, LD_{50} of the studied drug was 11,500 mg/kg. When applied locally to the skin of animals, LD_{50} of Neostomosan exceeded 10,000 mg/kg. The cumulation coefficient of Neostomosan was 2.9, which allows it to be classified as a group of drugs that do not have cumulative properties. A single treatment of calves with 0.3% of the drug did not cause toxicosis. After using Neostomosan in the specified concentration, a reliable change in biochemical parameters was noted in the blood of calves. At the same time, the studied drug did not cause changes in the internal organs of the calves.

Conflicts of Interest: The authors declare no conflict of interest.

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