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Studying of Toxicological Properties of the "Nb" Connection Possessing Antiparasitic Action.

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ABSTRACT

Parameters of acute and chronic toxicity of the "nb" connection having antiparasitic activity are studied. At a research of acute toxicity 60 males of white mice and 36 males of white rats have been used. Animals in 12 hours prior to experience didn't receive sterns. During experience they have been divided into 6 groups. The quantity of mice in one group was 10, and rats — 6. The studied connection has been entered by an animal inside by means of the probe. Rats and mice of the first group received substance in a dose of 400 mg/kg, the second — the 800, third — the 1200, fourth — 1600, and a heel — 2000 mg/kg. Animals of the sixth group served as control, they received inside vegetable oil. The volume of the entered solution for mice was 1 ml, for rats — 5 ml (at a research of acute toxicity) and 3 ml (at a research of chronic toxicity).During experiences death of animals wasn't registered. At a research of acute toxicity the dose of the studied connection which is most entered to mice and rats has made 2000 mg/kg. In this dose the "nb" connection has no toxicity that allows to refer it to the IV class of danger (substances low-dangerous).When studying chronic toxicity it is established that at prolonged use in doses of 1/10 and 1/20 parts from maximum (2000 mg/kg), this connection causes reduction of quantity of lymphocytes, increase in activity of enzymes of aspartate aminotransferase, alaninaminotransferase, amylase, content of the general protein and general bilirubin in blood serum. Also reduction of activity of alkaline phosphatase and level of glucose is noted.

Keywords: toxicology, antiparasitic drug, drug properties, blood counts

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INTRODUCTION

Helminthiases of animals are widespread everywhere, some of them are the general for the person and animals. At the animals infected with helminthes the weakened posterity is born, the young growth lags behind in growth and development [1, 3, 4]. Helminthes, parasitize in various bodies and tissues of animals, cause irreversible changes. Helminthiases, along with economic damage, do harm and to human health [4, 12].

For treatment of the diseases caused by helminthes at animals widely use various anthelminthic medicines [7, 11]. Many of them possess a broad spectrum of activity against trematodes, cestodes and nematodes. However all of them have a certain toxicity and can exert negative impact on an organism of an animal [7, 10].

Many anthelmintics have property to suppress immunity. Besides, at prolonged use of medicine the resistance of activators develops and its efficiency decreases [3, 8, 12].

One of the main requirements for anthelmintics is the selective toxicity for parasites and low toxicity for owners [2, 10].

It should be noted that the right choice of composition of active ingredients and a dosage form of anthelminthic medicine are important criterion of increase in efficiency of expulsion of helminthes as well criterion of decrease in toxicity [1, 7].

Now, despite synthesis of new antiparasitic connections and replenishment of the market medicines of foreign production, the livestock production feels the need on safe and highly effective, economically justified antiparasitic substances [9, 12]. Thus, development of the new, highly effective anthelminthic medicines possessing a broad spectrum of activity and high safety is a relevant problem of modern veterinary science [4, 8, 11].

Research objective I was studying of acute and chronic toxicity of the new "nb" connection having antiparasitic activity.

MATERIALS AND METHODS

The studied connection isn't dissolved in water, dissolved in organic solvents (in vegetable oil, air). On appearance represents white powder. Work was performed in vivarium of the Kazan state academy of veterinary medicine. At the same time were guided by methodical instructions by determination of toxic properties of the medicines applied in veterinary science and livestock production [5] and the guide to experimental (preclinical) studying of new pharmacological substances [6].

In the experiment on studying of acute toxicity used 36 males of white rats and 60 males of white mice, identical age with a body weight of 200-220 and 18-21 g respectively. Before statement of experience of animals maintained within 12 hours on a hungry diet. During experience of rats and mice have divided into 6 groups on 6 (rats) and 10 (mice) of individuals in everyone. As solvent of the studied substance applied vegetable oil. This connection was entered via the probe into a stomach in the form of oil solution of 5,0 ml — to rats, 1,0 ml — to mice. Rats and mice of the first group received the"nb" connection in a dose of 400 mg/kg, the second — the 800, third — the 1200, fourth — the 1600, fifth — 2000.The sixth group of animals was control, to them set inside vegetable oil in a dose of 5,0 ml (rat) and 1,0 ml (mouse).

Rats and mice were fed in 4 hours after introduction of the studied connection, the watering place wasn't limited. Within 14 days observed animals. Paid attention to appearance, behavior, a condition of indumentum and visible mucous membranes, existence of food excitability, mobility, a breath rhythm, time of emergence and the nature of intoxication, its weight and reversibility.

In the experiment on studying of chronic toxicity of the "nb" connection used 40 white rats of both sexes, with a body weight of 150-180 g. During experience of animals have divided into 4 groups on 10 rats in everyone. The chronic toxicity of the "nb" connection was studied in three doses. The first dose was close to

therapeutic and has made 20 mg/kg. The second and third doses have made 100 and 200 mg/kg (1/10 and 1/20 parts from most entered dose). This connection was entered in the form of solution on vegetable oil directly in a stomach by means of the probe. The volume of the entered solution I have made 3 ml. To rats of the first group entered the "nb" connection in a dose of 20 mg/kg, the second — 100 mg/kg and the third — 200 mg/kg within 5 days according to the guide to experimental (preclinical) studying of new pharmacological substances [5] once a day. The fourth group of animals served as control, they received orally directly in a stomach vegetable oil in a dose of 3 ml. Rats were fed 4 hours later after connection introduction. During experience observed animals. For the sixth day of all rats subjected euthanasias by method of a decapitation and took samples of blood from a jugular vein for carrying out hematological and biochemical researches.

Hematological researches were conducted in a hematology analyzer«Oka», calculation of quantity of leukocytes was carried out by means of Goryaev's camera. At a biochemical research of tests of serums of blood used the biochemical Mindray BS-200 analyzer, the TruCal calibrator of Dias and DDS reagents.

Conducted also opening and a path anatomical research of corpses of rats. Statistical processing of digital material was carried out on the computer with use of the editor of the spreadsheets Microsoft Excel.

RESULTS AND DISCUSSION

When studying acute toxicity of the "nb" connection the following results have been received. After introduction of the studied connection at all animals short-term oppression which disappeared in 2 hours was noted. With the expiration of this time of a rat and mouse willingly ate a forage, adequately reacted to sound, tactile and light irritants. The condition of an integument and visible mucous membranes was without pathological changes.

For studying of a pathanatomical picture carried out euthanasia and opening on one rat and two mice from each group. When opening noted stagnant hyperemia of intestines, liver, kidneys, small sites of granular dystrophy in a liver.

The dose defiant death of 50% of skilled animals of the "nb" connection hasn't been calculated as there was no death of skilled animals. Most entered dose has made 2000 mg/kg.

Thus, results of experiment on studying of acute toxicity of the "nb" connection have shown that it has no toxic property and belongs to the fourth class of danger (substances low-dangerous — (State Main Standard Requirements 12.1. 007-76).

When studying chronic toxicity of the "nb" connection the following results have been received. After introduction of the studied connection at rats no deviations of a clinical state were noted. As well as rats of control group, they willingly accepted a forage, fully reacted on external irritants.

Results of hematological and biochemical researches of tests of blood of animals are presented in tables 1 and 2.

From table 1 it is visible that before introduction of the "nb" Blood sedimentation rate (BSR) connection at rats of the first group I have made 1,09±2,26 mm/hour, hemoglobin level — 155,6±0,16 g/l, quantity of erythrocytes — 8,56±0,71 million / mkl, platelets — 524,6±0,08 1000/mkl, the percentage of monocytes — 1,6±2,26, lymphocytes — 57,6±0,47, eosinophils — 0,4±0,07, basophiles — 0,2±0,03, the maintenance of leukocytes — 10,5±0,77 1000/mkl. At rats of the second group these indicators were 1,12±0,39, 152,1±0,72, 8,48±0,24, 522,3±0,16, 1,8±0,03, 42,9±0,71, 0,5±0,29, 0,5±0,28, 10,3±1,06; at animals of the third group — 1,25±2,26, 145,5±0,17, 8,28±0,74, 545,9±0,1, 6,3±0,99, 41,8±0,32, 0,6±0,07, 0,6±0,02, 10,6±1,27 respectively. At rats of control group these parameters equaled 1,02±0,06, 158,5±0,42, 9,72±0,12, 629,4±0,48, 1,1±0,37, 62,4±0,09, 1,2±0,33, 8,3±0,13 respectively. At animals of the fourth group basophiles haven't been revealed. At rats of the third group the quantity of monocytes was 6,3±0,99%, against 1,1±0,37% at animals of control group. At rats of the second and third groups have established reduction of quantity of lymphocytes in blood. It demonstrates that at prolonged use in doses of 100 and 200 mg/kg this connection, apparently, exerts negative impact on function of immune system of an organism.

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Table 1: Hematological indicators of rats after introduction by him within 5 days of the "nb" connection

Num-ber of group	Blood sedimen-tation rate (BSR), mm/hour	Hemo-globin, g/l	Erythro-cytes, million/mkl	Platelets, 1000/mkl	Monocy-tes, %	Lympho-cytes, %	Leukocy-tes, 1000/mkl	Eosino-phils, %	Baso-philes, %
1	1,09±2,26	155,6±0,16	8,56±0,71	524,6±0,08	1,6±2,26	57,6±0,47	10,5±0,77	0,4±0,07	0,2±0,03
2	1,12±0,39	152,1±0,72	8,48±0,24	522,3±0,16	1,8±0,03	42,9±0,71	10,3±1,06	0,5±0,29	0,5±0,28
3	1,25±2,26	145,5±0,17	8,28±0,74	545,9±0,1	6,3±0,99	41,8±0,32	10,6±1,27	0,6±0,07	0,6±0,02
4	1,02±0,06	158,5±0,42	9,72±0,12	629,4±0,48	1,1±0,37	62,4±0,09	8,3±0,13	1,2±0,33	0
Physiological norm									
	1-3	120-180	5,3-10	500-1370	1-5	55-97	2,1-19,5	0-5	0-1

Table 2: Biochemical indicators of serum of blood at rats after introduction by him within 5 days of the "nb"connection

Num-ber of group	Urea, mmol/l	Creatinine, µmol/l	Glucose, mmol/l	Alanine-amino-transfe-rase, U/L	Aspartate amino-transfe-rase, U/L	Amylase, U/L	Alkaline phosphor-tase, U/L	Total protein, g/l	General bilirubin, mmol/l
1	5,09±1,1	77,2±0,31	8,93±0,89	85,5±0,22	107,1±0,21	596,3±0,03	1073,8±0,2	51,5±0,21	1,22±1,01
2	5,12±0,85	79,4±0,67	6,81±0,48	125,7±0,79	186,4±0,12	2790,0±0,54	258,1±0,27	91,6±0,35	6,54±0,93
3	7,29±0,58	85,6±0,19	6,56±0,19	187,4±0,13	207,6±0,43	2827,2±0,07	393,0±0,11	91,9±0,23	6,73±0,42
4	3,1±0,76	72,5±0,28	9,02±0,51	97,1±0,68	102,8±0,28	493,7±0,31	1082,4±1,09	54,2±0,85	1,42±0,35
Physiological norm									
	2,5-8,3	68-104	8,8-16,3	51-124	98-110	489-609	1066-1220	36-62	0-1,67

Note: 1 group-20 of mg/kg; 2 group-100 of mg/kg; 3 group-200 of mg/kg; 4 group control.

Biochemical indicators of serum of blood of skilled and control groups of rats are presented in table 2. From the table it is visible that concentration of urea in blood serum at rats of the first group has made 5,09±1,1 mmol/l, creatinine — 77,2±0,31 μmol/l, glucose — 8,93±0,89 mmol/l, activity of enzyme alaninaminotranferase— 85,5±0,22 U/l, aspartataminatransferase — 107,1±0,21 U/l, amylases — 596,3±0,03 U/l, alkaline phosphatase — 1073,8±0,2 U/l, the content of the general protein — 51,5±0,21 g/l, the general bilirubin — 1,22±1,01 μmol/l. At animals of the second group these parameters equaled 5,12±0,85, 79,4±0,67, 6,81±0,48, 125,7±0,79, 186,4±0,12, 2790,0±0,54, 258,1±0,27, 91,6±0,35, 6,54±0,93 respectively. At rats of the third group they have made 7,29±0,58, 85,6±0,19, 6,56±0,19, 187,4±0,13, 207,6±0,43, 2827,2±0,07, 393,0±0,11, 91,9±0,23, 6,73±0,42, and the fourth — 3,1±0,76, 72,5±0,28, 9,02±0,51, 97,1±0,68, 102,8±0,28, 493,7±0,31, 1082,4±1,09, 54,2±0,85 and 1,42±0,35 respectively.

At rats of the second and third groups increase in activity of alaninaminitransferase enzymes (125,7±0,79, 187,4±0,13), aspartataminotransferase(186,4±0,12, 207,6±0,43 U/l) and amylases (2790,0±0,54, 2827,2±0,07 U/l) noted. Content of alkaline phosphatase at them, on the contrary, has been lowered (258,1±0,27, 393,0±0,11 U/l). At animal these groups the content of the general protein and bilirubin increased and also concentration of glucose in blood serum decreased.

At a path anatomical research of corpses of rats of the second and third groups after five day introductions of the "nb" connection in doses of 100 and 200 mg/kg have established stagnant hyperemia and granular dystrophy of a liver, kidneys, small sites with dystrophic changes in a pancreas.

CONCLUSION

The acute and chronic toxicity of the "nb"connection at introduction inside is studied. It is established that in a dose of 2000 mg/kg it has no toxicity and belongs to the fourth class of danger. At introduction of the "nb" connection within 5 days in a dose of 100 and 200 mg/kg reduction of quantity of lymphocytes in blood is noted. Content of the general protein and bilirubin, activity of enzymes of aspartat aminotransferase, alanine aminotransferase, amylase increases, concentration of enzyme of alkaline phosphatase and glucose decreases. At a path anatomical research of corpses of rats venous stagnation in a liver, kidneys, granular dystrophy of these bodies and also the changed sites small dystrophic in a pancreas noted.

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