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Evaluation Of The Hexaaquahexaimidazole Copper Sulfate Drug Effectiveness In The Main Helminthiases Of Sheep

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Abstract

Anthelmintic drug hexaquahexamidazole sulfate copper (II) with low toxicity (LD_{50}) = 1580 mg/kg), has a specific cestodocidal activity of 100% in 1% concentration in "in vitro" experiments on echinococcal protoscolex (Echinococcus granulosus) shows high anthelmintic efficiency in a dose of 100-150mg/kg in sheep with manesiosis (extenefficacy and intenefficacy =100%). Determination of hexaquahexamidazole sulfate drug acute toxicity parameters was carried out on rats. This drug allows complex treatment and prevention of helminthiasis in sheep and restores animals' productivity in a short time.

Keywords: coordination compound, copper hexaquahexamidazole sulfate, pharmaco toxic property, anthelmintic drug, scolex, extenefficacy, intenefficacy.

1. Introduction

Helminthic diseases of farm animals (Bondarenko et al., 2022) are widespread in Kyrgyzstan and cause great economic damage to animal husbandry in the form of a significant decrease in the productivity and yield of all types of animals. According to the latest data (Arzybaev & Baydinov, 2015; Arkhipov, 2009; Belenky, 1963; Veselova, 1978; Demidov, 1982; Didenko, 1993; Isaev & Arzybaev, 2012), the prevalence of sheep moniesia and other intestinal helminths reaches 85-100%. Cases of human zoonotic diseases (alveococcosis, echinococcosis, intestinal helminthosis, etc.) also increased and significant centers of echinococcosis and alveococcosis were found in the Alai Valley, Osh region.

The implementation of the most promising biological, ecological methods and vaccinations in practice is difficult due to their high cost, still the main method of combating helminthiasis in animals is chemotherapy with the use of highly effective, low-toxic anthelmintics of a wide spectrum of action, safe for the animal organism and the environment (Arkhipov, 2004). In veterinary medicine, albendazole, fenbendazole, mebendazole, fenasal, etc. are used for the treatment of helminthiasis with drugs in its pure form and with various compositional additives (Oripov et al., 2019).

In the process of practical use, along with positive anthelmintic properties, they also have significant disadvantages such as low solubility, high toxicity (Patent KR, 2017) and low efficiency (Patent KR, 2022), complexity and high cost of drug synthesis (Patent KR, 2020a; Patent KR, 2020b). On the other hand, frequent use of the same drugs for deworming animals leads to the emergence of resistant strains of helminths (Karmaliev, 2013).

To increase the water solubility, reduce the toxicity and increase the efficiency of the preparations, there are well-known physical and chemical methods of modifying the properties of these substances. We propose a method of

solubility, with the help of which it is possible to obtain coordination compounds with increased solubility and low toxicity.

Based on the above, the search for a new anthelmintic drug and the study of its pharmaco-toxicological properties is an urgent task in the fight against animal helminthiasis.

The coordination compound hexaquahexamidazole copper sulfate [Cu $(C_3H_4N_2)6]$ $SO_4 \cdot 6H_2O$ was synthesized by the interaction of imidazole with copper sulfate by the solubility method at 25°C. The composition, properties and the compound with structure [Cu $(C_3H_4N_2)6]$ $SO_4 \cdot 6H_2O$ were characterized by physical and chemical analysis methods (elemental, DTA, RFA, RSA and IR spectroscopy) (Malabayeva et al., 2016; Malabayeva et al., 2018). Previously, synthesis and crystal structures of pyridoxine with manganese and cadmium sulfate coordination compounds were reported (Furmanova et al., 2009; Furmanova et al., 2011).

This research purpose is to study the acute toxicity and anthelmintic efficiency of new drug based on hexaquahexamidazole sulfate.

2. Research methods and materials

Synthesis of hexaquahexamidazole sulfate compounds. For this purpose, 1.34 g (0.01 mol) of copper sulfate was dissolved in 50 ml of distilled water, then 4.0848 g (0.06 mol) of imidazole was add while stirring. Resulting solution was heated up to 50°C with intense stirring and slowly evaporated in a water bath until its volume reduced to 1/3, then reaction mixture was cooled and left for crystallization. Precipitated light blue crystals washed and dried to reach constant mass. The total yield of product is 4.407 g, which is 81.23%.

Copper hexaaquahexamidazole sulfate $[CuSO_4 \cdot 6C_3H_4N_2 \cdot 6H_2O]$ is a light blue crystal, odorless, stable in air, well soluble in water, alcohol, poorly in acetone, chloroform, practically insoluble in benzene and carbon tetrachloride. Molecular weight is 673.089 g/mol, relative density is 1.83 g/cm^3.

The experiment to determine hexaquahexamidazole sulfate drug acute toxicity parameters was carried out on 36 clinically healthy rats with a live weight from 18 to 20 grams by oral administration of substance in the form of a 10% solution using a syringe equipped with a special metal probe in a dose of 800, 1200, 1600, 2000 and 2400 mg/kg body weight. Control animals received the corresponding volume of physiological NaCl solution. Statistical processing of digital materials was carried out using the method (Litchfield & Wilcoxon, 1949) in modification (Roth, 1960) using conventional graph paper (Kudrin & Ponamareva, 1967).

3. Results and discussion

The results of pharmaco-toxicological studies showed that the maximum tolerated dose (LD_0) of hexaquahexamidazole sulfate copper for white mice was 800 mg/kg, LD_{16} is 1230 mg/kg, the average lethal dose LD_{50} is 1580 mg/kg, LD_{84} is 2150 mg/kg and absolute lethal dose (LD_{100}) is 2400 mg/kg as shown in Table 1.

As can be seen from the analysis of the results of statistical processing, the average lethal dose (LD_{50}) of hexaquahexamidazole copper sulfate for white mice when administered orally exceeds 1000 mg/kg. This means that according to the existing classification of the chemotherapeutic drugs danger according to the degree of impact on the body (GOST 12.1.007-76), tested compound belongs to moderate danger low-toxic chemical substances (Sanotsky & Ulanova, 1976).

Table 1. Parameters of hexaquahexamidazole sulfate acute toxicity in rats after oral administration.

Dose, mg/kg	**Quantity**	**Results**	Acute toxicity parameters at P=0.05

		Dead	Survived	
800	6	0	6	LD_0=800 mg/kg
1200	6	1	5	LD 16=1230 mg/kg
1600	6	3	3	LD_{50} =1580 mg/kg
2000	6	5	1	LD_{84} = 2150 mg/kg
2400	6	6	0	LD_{100} = 2400 mg/kg
Control	6	0	6	

The test of anthelmintic activity of hexaquahexamidazole sulfate was carried out in experiments in vitro (out of body), while we studied the cestodocidal activity of the compound, i.e. effect on cestodes (tapeworms). New substance testing for cestodocidal activity was performed on protoscolex from fresh echinococcal cyst (Echinococcus granulosus). For their experiments, echinococcal bubbles were obtained from the affected organs (liver, lungs) of sheep, which were killed in slaughterhouses. They were immediately placed in saline solution containing NaCl at room temperature. Before the experiment, the echinococcal blister was opened, together with the liquid, necessary amount of protoxolex was taken and placed in a Petri dish, and then test compound was added in the form of a 1% aqueous solution. For comparison, imidazole, albendazole and copper sulfate were taken in full or concentrated form (albendazole suspension). As a control, a Petri dish with sodium chloride solution without adding any preparations was placed approximately the same amount of protoscolex.

Then 2-3 drops of 1% aqueous solution of eosin were added to experimental and control cups and left for 16-18 hours at room temperature. It is known that dead tissues are stained with eosin, and living tissues are not (Krotov, 1961). The next day, under a binocular microscope (MBS-1), the number of dead and alive protoscolex was counted in all the cups. Comparing the results, the degree of anthelmintic activity was established, which was expressed in percentages.

The obtained results of experiments show (Table 2) that hexaquahexamidazole sulfate copper is in the tested concentration of protoscolex Ech. granulosus has a destructive effect. In fact, at 1% concentration, 100% death of protoscolex occurs.

Table 2. Cestodocidal activity of hexaquahexamidazole sulfate during in vitro experiments.

No	**Compound**	**Compounds' concentration**	**Protoscolex quantity Ech. granulosus**			**Efficiency, %**
			Total	Alive	Dead	
1	Hexaaquahexaimidazole copper sulfate	1%	47	0	47	100
2	Imidazole	1%	52	34	18	47.1
3	Albendazole $C_{12}H_{15}N_3O_2S$	1%	38	22	16	42.1
4	Copper sulfate $CuSO_4$	1%	40	20	20	50.0

5	Control	1%	44	44	0	0

The anticestod efficiency of imidazole in the concentration was 47.1%, albendazole 42.1%, copper sulfate 50%, respectively. In the control cup, the death of protoscolex was observed.

Having obtained a positive result in in vitro experiments, we continued to study the anthelmintic effect of hexaquahexamidazole sulfate copper in in vivo experiments on helminthic sheep. Experiment was conducted according to the control-test method (World Association for the Advancement of Veterinary Parasitology, 1995), for this purpose in a private hospital Tagayberdieva A.K. 36 lambs born in the current year, spontaneously infested with cestodes of the genus Moniezia, with a live weight of 25-31 kg, were selected using double helminthoscopy by the method of flotation with a saturated solution of ammonium nitrate (Kotelnikov & Khrenov, 1980). Animals were divided into 6 groups, according to the analogues principle, taking into account infection and weight. During the experiment lambs were in a common flock under constant observation. To the first group of animals was given 50 mg/kg hexaquahexamidazole copper sulfate water suspension orally in a bottle, and the fifth group copper sulfate in the form of a 1% aqueous solution of 25-30 ml per head. The sixth group was subjected to control and deworming of sheep.

On the 11th day, all animals were subjected to a general helmintocoproscopic examination. Calculations to determine the anthelmintic efficiency of tested preparations were also carried out by control test method, considering decrease in the intensity of eggs release. Experimental results are shown in Table 3, that out of 4 lambs that received copper hexaquahexamidazole sulfate at a dose of 50 mg/kg (group No. 1) were free from helminths-3 (EE=75%), in the second and third groups all lambs that received this drug in doses of 100 and 150 mg/kg isolated from cestodes (EE=100%). In the fourth group of animals dewormed with imidazole (20 mg/kg), only 2 out of 4 heads were cured (EE=16.7%). Sulfate copper showed 50% extensive effectiveness in moniesiosis in sheep.

Table 3. Test results of hexaquahexamidazole sulfate medicine in sheep moniesiosis.

No.	**Drug name**	**Dose, mg/kg**	**Animals total number**	**Results**		**Efficiency**	
				Number of treated animals	**Total number of monies in the group**	**EE**	**IE**
1	Hexaaquahexaimidazole copper sulfate	50	6	4	8	83.3	77.7
2	Hexaaquahexaimidazole copper sulfate	100	6	6	0	100	100
3	Hexaaquahexaimidazole copper sulfate	150	6	6	0	100	100
4	Imidazole	20	6	2	20	16.7	55.5
5	Copper sulfate	1% solution	6	3	19	50	52.7

		30 ml					
6	Control	-	6	0	36	-	-

Drug effectiveness was determined by the intensity of magnesium release from egg. It is established that intensity effectiveness of hexaquahexamidazole sulfate in a dose of 50 mg/kg is 66.6%, and in a dose of 100 and 150 mg/kg it is 100%. Imidazole and copper sulfate in tested doses showed intensive efficiency equal to 55.5 and 61.1%, respectively.

Compared to other known drugs, this drug has the following advantages: high anthelmintic efficiency in monies infection, simple production technology, low toxicity (class III) and low cost. The use of drug will allow to carry out complex treatment and prevention of helminthiasis in sheep and restore productivity of animals in a short period.

Conclusion

In testing the anthelmintic activity of new synthetic compound hexaquahexamidazole sulfate in vitro experiments on echinococcus protoscolex (Echinococcus granulosus), it was established that it has high cestodocidal activity (100%), low toxicity (LD_{50} = 1580 mg/kg). This compound can be used as an anthelmintic drug to combat cestodoses in animals. No less important advantage of proposed hexaquahexamidazole sulfate copper is the availability of raw materials, prostate synthesis, high yield of the target product (81.23%) and low cost.

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