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EFFICIENCY OF THE IODINE-CONTAINING COMPLEX AGAINST AVIAN INFLUENZA A VIRUS SERIES

Abstract. The paper contains materials on the study of exposure to avian influenza A virus iodine-containing complexes synthesized in the JSC "Scientific Center of Anti-Infectious Drugs". Conducted screening of educed the most active iodine-containing complex from a nine investigational. It was found that the iodine-containing complex is a low-toxic compound and exhibiting antiviral activity against influenza A virus. It has a high potentiating effect when combined with commercial antiviral drugs.

Keywords: iodine-containing complexes, cytotoxicity, acute toxicity, influenza viruses, antiviral activity, potentiating action.

Introduction. The problem of improving systems of ant epizootic measures for the prevention and treatment of infectious diseases has always been and remains very relevant. According to the literature, an analysis of the epizootic situation shows that in recent years there has been an increasing threat of widespread infectious diseases throughout the world, which brought a significant economic damage and posing a danger to human infection. Among viral diseases, bird flu comes first. This virus - refers to a highly contagious and widespread viral infection. The causative agent of the infection - the influenza virus is divided into three serological types: A, B and C. Type A viruses causes diseases in animals and humans [1-6].

Currently, bird flu remains a global problem in veterinary medicine. According to the World Organization for Animal Health (OIE), in 2018 507 foci of highly pathogenic avian influenza were registered in 35 countries such as Afghanistan (3), Bangladesh (2), Bulgaria (25), United Kingdom (21), Vietnam (10), Germany (5), Denmark (30), India (11), Iraq (15), Cambodia (7), China (13), Russia (82), Saudi Arabia (22), Sweden (11), Japan (14) and etc. The virus have been reported in herds of agricultural and wild bird populations in 15 countries in Asia, Africa, Europe and United States. There were registered cases of highly pathogenic influenza in the world: H5N1 (17 countries), H5N2 (3 countries), H5N5 (Germany, Holland, Montenegro), H5N6 (5 countries), H5N9 (France), H7N7 (Italy), H7N1 (Algeria). The most propagation is the H5N6 influenza virus, as well as similar viruses of the subtypes H5N8, H5N1. Seasonal migrations of birds from Southeast Asia to the Russian Federation during the nesting period are the main prerequisites for the possibility of influenza infection of poultry in industrial bird's farms and private farmsteads. The likelihood of a bird flu virus entering the territory of Kazakhstan with migratory flows of migratory birds remains quite high. According to the WHO experts, every year from 3 to 5 million people become ill with severe forms of influenza and from 250,000 to 500,000 people die around the world (Uchaykin V.F., 2004, I. Toshihiro, 2001).

Despite this, the practice of modern veterinary medicine uses a relatively small set of drugs with a wide range of antiviral effects. The emergence of drug resistance in viral pathogens to antiviral drugs leads to a decrease or complete loss of the effectiveness of the therapy and the flightiness of spread of infection goes down, this leads with searching of new drugs (Sidorenko S.V., 2004, Abraham EP, 1940, Walsh C. 2000). One of the main directions in the development of antiviral drugs is the synthesis of

analogues from known drugs. So iodine-containing drugs widely used in veterinary medicine and medicine, determine high biological activity and versatile pharmacological action, without causing resistance in the pathogen [7-10]. This work will present the results of studies, which will indicate new synthesized iodine – containing complexes for avian influenza virus type A [11, 12].

Methodology and research method. For the research were used: transplantable cell line MDCK (Madin-Darby canine kidney); - seven-day-old chickens weighing 250-350 g, - sexually mature outbred mice of both sexes, weighing 18-24 g, rats weighing 160-230 g, 7-18 day old developing chicken embryos. During the conducting of research the following strains of influenza viruses were used: - Waybrige strain A/78, (H7N7)/FPV /; Rostock strain A/34, (H7N1), /FPV/black laughter strain A, Atyrau 744/04, (H13N6); Almaty strain A 5/98, (H3N2).

Cell and virus types were cultured in a monolayer using DMEM medium and 10% cattle serum was added (Applichem, Sigma, USA). The propagation of influenza viruses was carried out on seven to nine chicken embryos by introducing 0.2 ml of the virus into the allantois cavity and on the MDCK cell culture by infection of the cell monolayer. Infectious virus titer was determined in a hem agglutination reaction (RGA according to Reed & Muench).

Results of own research. A study was made of 9 iodine-containing α -dextrin complexes capable of penetrating the membrane and nucleus of a microbial cell. The molecular iodine of the active drugs' complex compound is located inside of the α -dextrin helix and is coordinated by alkali metal halides and polypeptides. In this structure, molecular iodine difficulty to interact with blood components and reaches the pathogen cell unchanged, because only nucleotides of the DNA of a pathogen can compete with polypeptides for complexation with iodine. The biopharmaceutical solubility of the iodine-containing complexes which were taken for the study was researched in the pH range of 1.2; 4.5; 6.8 corresponding to physiological fluids of the gastrointestinal tract. The determination of the permeability of iodine-containing complexes was carried out on an MDCK cell culture. In 96 well plates with MDCK cell culture, 0.2 ml of DMEM medium containing the test substances at a concentration of 0.5 mg / ml was added. The results of studies of the active complex substance's ability to dissolve when applied internally are presented in figures 1.

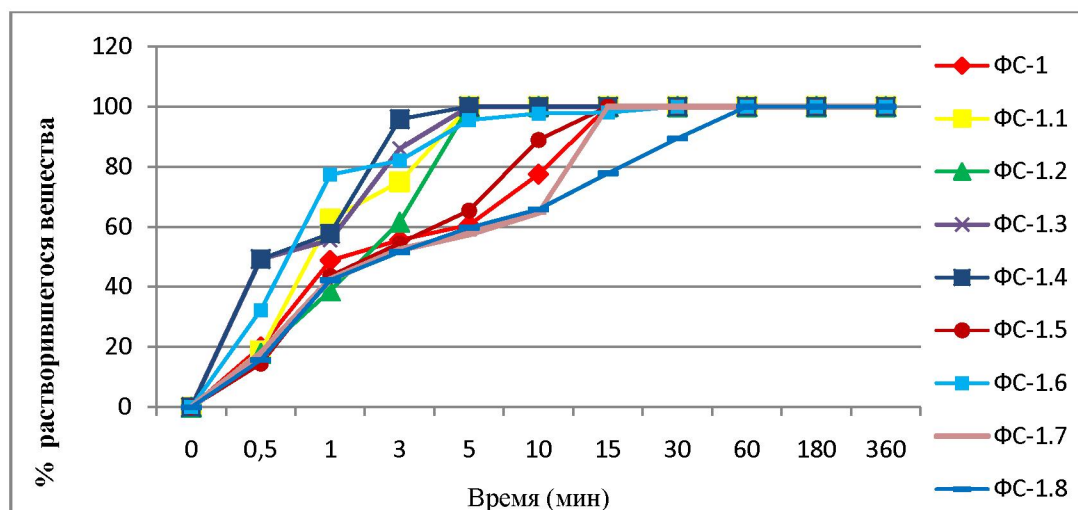


Figure 1 – Pharmacopoeia solubility of FS-1 in water, a buffer solution with a pH of 1.2, 4.5 and 6.8

According to the results of the studies, it was found that the biopharmaceutical properties of all iodine-containing complexes based on the degree of their solubility in water, buffer solutions with a pH of 1.2; 4.5; 6.8 and permeability in an experiment on an MDCK cell culture corresponded to class III bioequivalence of drugs. The studied complexes divided by substances with "high" solubility and "low" permeability.

The safety of iodine-containing complexes was determined on an MDCK cell culture by the MTT test and by using concentrations from 0.03 to 25.0 mg/ml.

Although, an experiment in mice by determining toxicological studies of the maximum tolerated doses of the active substance from 0.6 - 13.4 ml/kg. The results of the MTT test on the MDCK cell line for iodine-containing complexes are shown in figures 2 and 3. The results of acute toxicity of iodine-containing complexes in an experiment in mice with intraperitoneal administration are shown in figure 4.

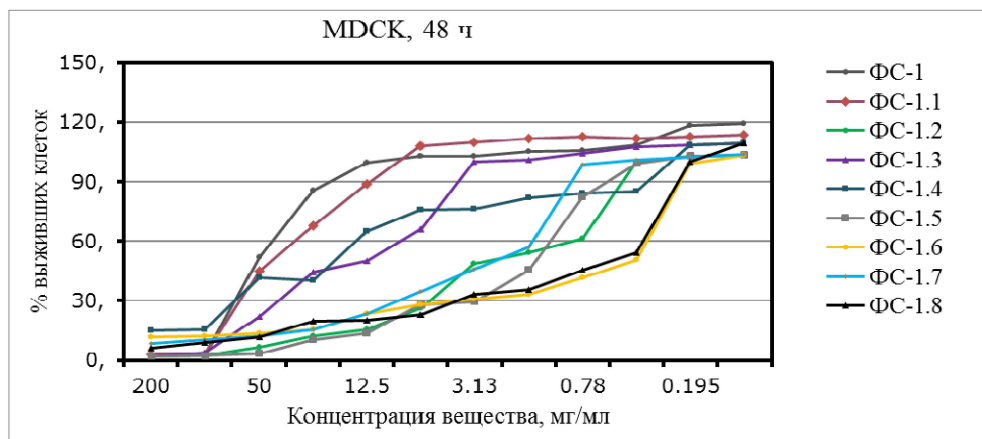


Figure 2 – The effect of iodine-containing complexes on the survival of the MDCK cell line after 48 hours of incubation

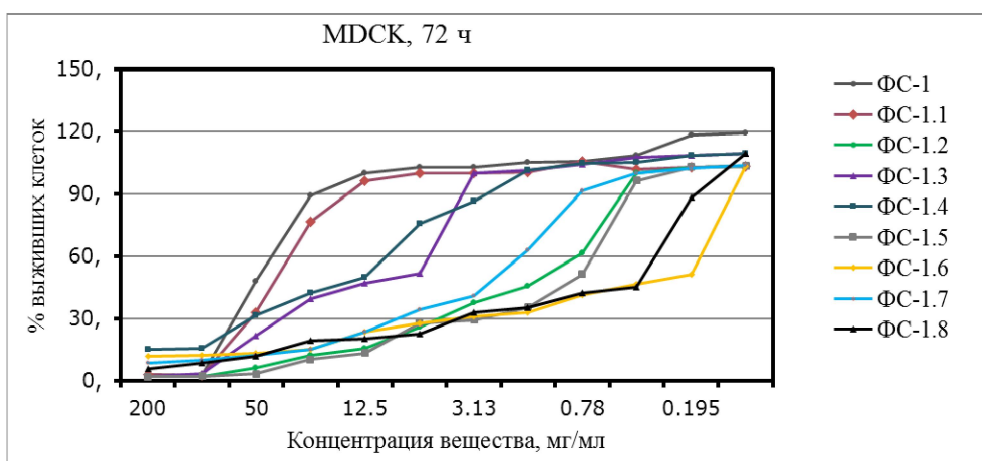


Figure 3 –The effect of iodine-containing complexes on the survival of the MDCK cell line after 72 hours of incubation

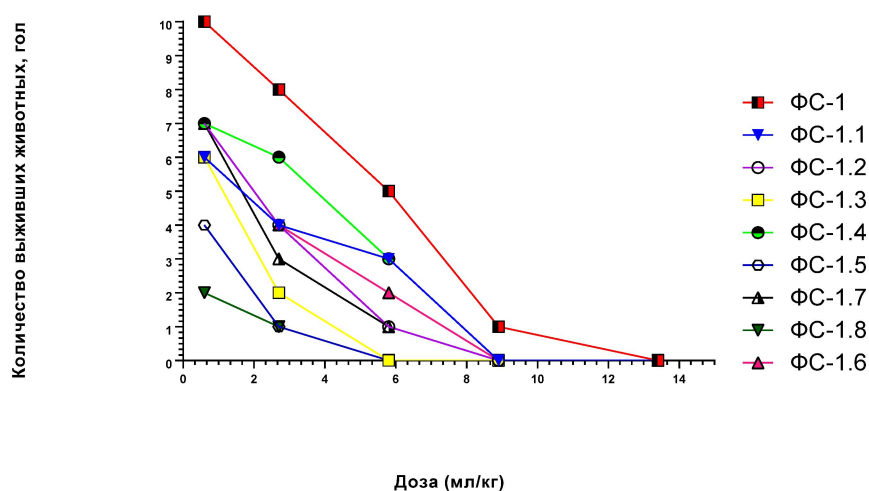


Figure 4 – Determination of FS-1's acute toxicity in an experiment in mice with intraperitoneal administration

As shown in figure 2 and figure 3 iodine-containing complexes FS-1, FS-1.1, FS-1.3, FS-1.4 have a low toxic. The CC_{50} of complexes FS-1, FS-1.1, FS-1.3, FS-1.4 after 48 hours of cultivation was in the concentration range from 10.0 to 50.0 mg/ml and after 72 hours of cultivation was in the concentration range from 5.0 to 50.0 mg/ml. Toxicity did not increase depending on the duration of cultivation. The iodine-containing complexes FS-1.2, FS-1.5, FS-1.6, FS-1.7, FS-1.8 had moderate toxicity. Their CC_{50} ranged from 0.39 to 2.13 mg/ml after 48 hours of incubation on an MDCK cell culture and from 0.20 to 1.77 mg/ml after 72 hours.

Determination of acute toxicity in mice from Figure 4 showed that all the iodine-containing compounds FS-1.1, FS-1.2, FS-1.3, FS-1.4, FS-1.5, FS-1.6, FS-1.7, FS-1.8, except FS-1, in doses of 0.6 up to 13.4 ml/kg caused a pronounced toxic effect. At the same time, the FS-1 complex under study showed the least toxic properties in doses from 0.6 to 5.8 ml/kg.

An in vitro study of the antiviral effect of iodine-containing compounds was carried out using the Waybrige/78 strain A virus (H7N7) as an example. It was found that the reproduction of influenza A virus on an inoculated cell culture of MDSK was suppressed under the influence of complexes FS-1.1, FS-1.2, FS-1.3, FS-1.5, FS-1.6, FS-1.8 in comparison with the control titer by no more than 2.0 log. At the same time, the FS-1.4 complex reduced the titer of the virus by 5.0-6.0 log in comparison with the control. The greatest antiviral effect among the studied iodine-containing complexes was found in the FS-1 and FS-1.7 complexes, which reduced the virus titer by 6.0-8.0 log. However, the FS-1.7 complex showed high toxicity in further experiments on animals. Therefore, as a result of studies of iodine-containing complexes based on physicochemical properties, determination of cytotoxicity in an MDCK cell culture, acute toxicity in an experiment in mice, antiviral activity using the example of influenza virus strain A Waybrige/78, (H7N7), in vitro, showed the most effective composition of the iodine-containing complex FS-1. Our further studies were aimed at studying the antiviral activity of the iodine-containing complex FS-1 against avian influenza A virus in experiments on chicken embryos, chickens, as well as determining the potentiating effect in experiments in vitro, in ovo, in vivo.

By determining the safety of the FS-1 complex in mice, an accumulation coefficient of 1.45 was calculated, and the absence of chronic toxicity of the FS-1 complex with repeated administration (24 times) to animals was established.

Studies of the determination of acute toxicity in rats showed that oral administration of FS-1 made it possible to calculate active doses effective in 50% of cases and toxic (lethal) doses equal to LD_{16} - 571 mg/kg, LD_{50} - 922 mg/kg, LD_{84} - 1273 mg/kg, LD_{100} - 1449 mg/kg.

According to the toxicity scale of substances administered per os (Hodg G., Gleason S., 1975; S. A. Kutsenko, 2002) the studied complex FS-1 can be attributed to low-toxic substances of the third class of biological safety.

The study of embryo toxicity of FS-1 was carried out on 10, 12 and 18 day old chicken embryos. Into the chorion-allantois cavity 0.2 ml of the studied concentrations of FS-1. Based on the data obtained, it was found that the studied FS-1 in concentrations from 0.8 to 1.6 mg/ml did not have a toxic effect. In concentrations from 3.3 to 6.6 mg/ml, 10 % to 20 % of embryos died. The embryo toxic effect of FS-1 is expressed in concentrations from 13.1 to 52.5 mg/ml, where from 50 % to 100 % of chicken embryos died. It is also shown that the embryo toxicity of FS-1 depends not only on its concentrations, but also on the timing of development of the chicken embryo. With the introduction of FS-1 to embryos with a developmental period of 10; 12 and 18 days at a concentration of 52.5 mg/ml killed 100 %, 80 % and 50 % of chicken embryos, respectively. With the introduction of FS-1 at a concentration of 26.3 mg/ml, 80% of chicken embryos died with a development period of 10 days, 60 % of 12-day-old chicken embryos and 10 % of 18-day-old chicken embryos.

The lowest toxic concentration that caused the death of 70 % - 10-day-old embryos is 13.1 mg/ml. With the introduction of FS-1 at a concentration of 26.3 mg/ml, 80 % of chicken embryos died with a development period of 10 days, 60 % of 12-day-old chicken embryos and 10% of 18-day-old chicken embryos. The obtained concentration of toxicity of FS-1 in an experiment on embryos was used to study antiviral activity. The study of acute toxicity of FS-1 was carried out in an experiment on seven-day-old chickens. Studies have shown that - the dose of LD_{50} for FS-1 was 52.5 mg/kg when administered orally and subcutaneously to the chicken. Medium toxic doses were established with subcutaneous administration of FS-1 in concentrations from $1/8 LD_{50}$ to $1/2 LD_{50}$ mg/kg. At the same time, the tolerated

concentration of the antiviral drug named «remantadine» (control substance) was fixed at a dose of 8.33 mg/kg.

Studies of the antiviral activity of FS-1 were measured in experiments with influenza viruses with various strains: strain A Rostock / 34, (H7N1), black-headed laughter, strain A Atyrau 744/04, (H13N6); Almaty strain A 5/98, (H3N2) on ten-day-old chicken embryos by neutralizing the virus at a dose of 100 EID₅₀ /0.2 ml with experimental concentrations. It was found that FS-1 at a concentration of 3.3 mg/ml suppressed the reproduction of viruses by 2.0 log, and at a concentration of 6.6 mg / ml completely suppressed 100 infectious doses of influenza A virus of different strains: strain A Rostock / 34, (H7N1); black-headed laughter, Atyrau strain A 744/04, (H13N6); Almaty strain A 5/98, (H3N2).

The determination of the antiviral therapeutic efficacy of FS-1 was carried out in an experiment on seven-day-old chickens against the influenza virus strain A/Rostock/34, (H7N1). Three doses of FS-1 were used in the experiment: 6.6 mg/kg, corresponding to 1/8 LD₅₀; 13.1 mg/kg corresponding to 1/4 LD₅₀ and 26.3 mg/kg corresponding to 1/2 LD₅₀. As a control, a commercial drug «remantadine» was used at a therapeutic dose of 8.33 mg/kg for comparison. The results of the study are presented in figure 5.

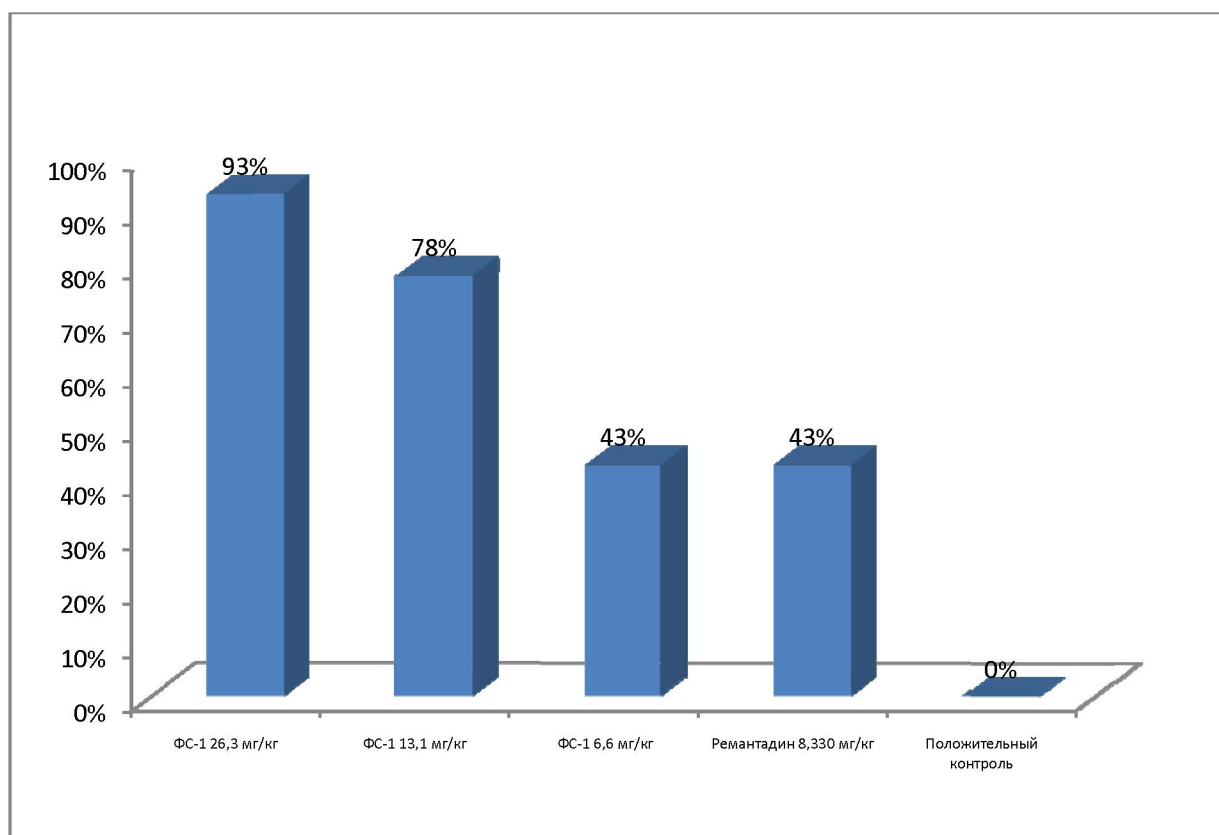


Figure 5 – The therapeutic effect of the drug FS-1

The results indicate that FS-1 showed high therapeutic efficacy at doses of 13.1 and 26.3 mg/kg against influenza virus in infected chickens. The use of the FS-1 complex as a therapeutic agent in doses of 26.3 and 13.1 mg/kg led to 93 % and 78 % survival of chickens, while the use of the drug «remantadine» in a therapeutic dose of 8.33 mg/ml retained only 43 % of the livestock.

The antiviral prophylactic efficacy of FS-1 was determined in an experiment on seven-day-old chickens against the influenza virus strain A Rostock / 34, (H7N1). In this case, four doses of FS-1 were used: 3.3 mg/kg corresponding to 1/16 LD₅₀; 6.6 mg/kg, corresponding to 1/8 LD₅₀; 13.1 mg/kg corresponding to 1/4 LD₅₀ and 26.3 mg/kg corresponding to 1/2 LD₅₀. The multiplicity of per os administration of the studied iodine-containing complexes and «remantadine» was seven days. As a control, a commercial drug «remantadine» at a dose of 8.33 mg/kg was used for comparison. The results of the study are presented in figure 6.

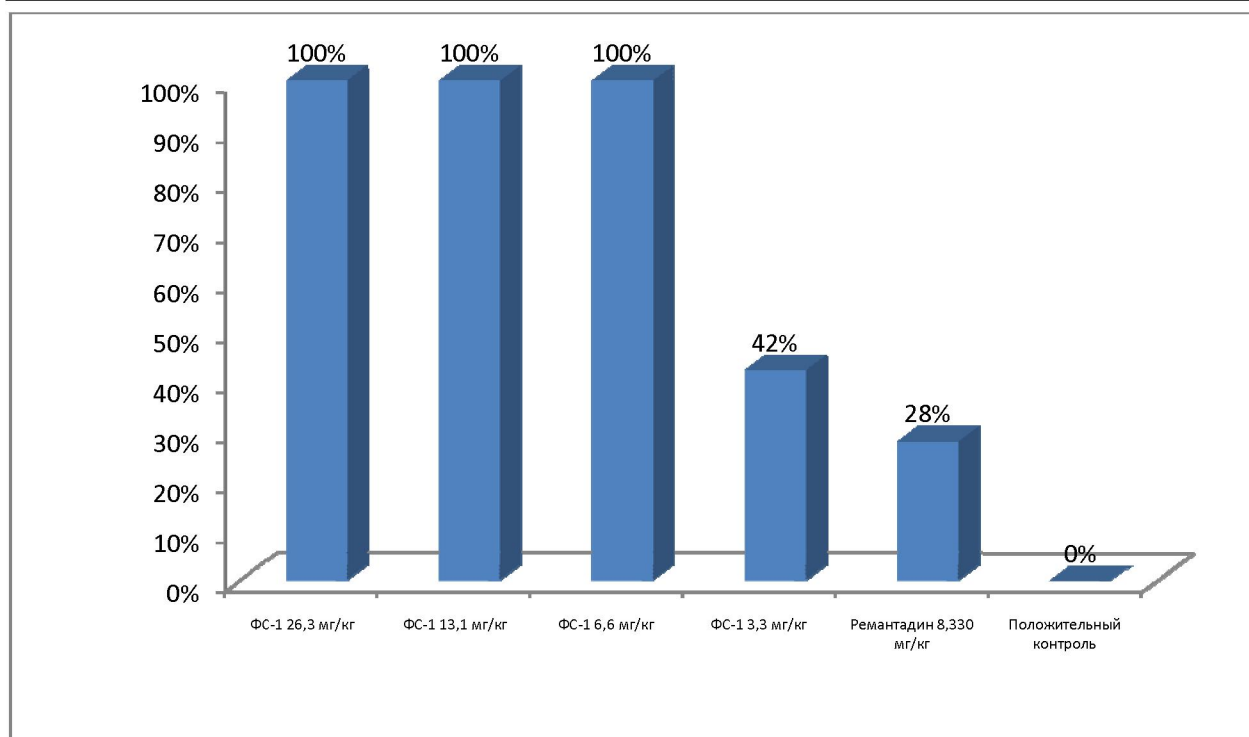


Figure 6 – The preventive effect of the drug FS-1's action

The results of studying the antiviral prophylactic effectiveness of FS-1 in experiments on chickens infected with Rostock/34 strain A influenza virus, (H7N1), showed pronounced prophylactic efficacy (100 %) in the studied doses of 26.3; 13.1 and 6.6 mg/kg more than three times the prophylactic effectiveness of the control antiviral drug «remantadine». However, at a dose of 3.3 mg/kg, the prophylactic efficacy of the investigated iodine-containing complex FS-1 is 1.5 times higher than the prophylactic dose of 8.33 mg/kg of the antiviral drug «remantadine».

In our experiments, we also established a significant pharmacological effect on influenza A virus. In various models with antiviral drugs widely used in practice, remantadine, oseltamivir, ribazole and amixin revealed a potentiation effect under the influence of the FS-1 iodine-containing complex. The results of potentiation with the combined use of the iodine-containing complex FS-1 with the studied antiviral drugs were accompanied by an increase in their therapeutic effect. Based on the results, we have compiled and filed an application for an invention with the patent department of NTsGNTS RK.

Thus, the studies and the results obtained allow us to conclude that the iodine-containing complex FS-1 is a low-toxic compound, exhibits antiviral activity against influenza A virus, and has a high potentiating effect when used in conjunction with commercial drugs. Further studies of the drug are actively continues.

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«Инфекцияға қарсы материалдардың ғылыми орталығы» АҚ, Алматы, Қазақстан

ҚҰСТАРДЫҢ А ТҰМАУЫНА ҚАРСЫ КҮРЕСТЕ ИОДТЫ ҚОСЫЛЫСЫНЫҢ ЭФФЕКТИВТІЛІГІНЕ БАҒА БЕРУ

Аннотация. Жұмыста «Инфекцияға қарсы дәрі-дәрмектердің ғылыми орталығы» АҚ-да синтезделген құрамында йод бар кешендерді зерттеуге арналған материалдар ұсынылған. Зерттелген зерттеуінде кешендер құрамында ең белсенді йод бар кешені анықталды. Алынған құрамында йод бар кешендердің құс тұмауы вирусына әсерін зерттеу нәтижелері сипатталған.

Түйін сөздер: құрамында йод бар кешендер, цитотоксикалық, жедел уыттылық, тұмау вирустары, вирусқа қарсы белсенділік, потенциалды әсер.

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ОЦЕНКА ЭФФЕКТИВНОСТИ ИОДСОДЕРЖАЩЕГО КОМПЛЕКСА В БОРЬБЕ С ГРИППОМ ПТИЦ А

Аннотация. В работе изложены материалы по изучению воздействия на вирус гриппа птиц А иодсодержащих комплексов, синтезированных в АО «Научный центр противоиных препаратов». Проведенный скрининг из девяти исследованных комплексов выявил наиболее активный иодсодержащий комплекс. Установлено, что иодсодержащий комплекс является малотоксичным соединением, проявляет противовирусную активность в отношении вируса гриппа А, обладает высоким потенцирующим действием при совместном применении с коммерческими противовирусными препаратами.

Ключевые слова: иодсодержащие комплексы, цитотоксичность, острая токсичность, вирусы гриппа, противовирусная активность, потенцирующее действие.

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