SUPPRESSION OF AVIAN INFECTIOUS BRONCHITIS VIRUS REPRODUCTION BY SOME PLANT EXTRACTS

ANNOTATION

A variety of variants of the avian infectious bronchitis virus creates certain difficulties in vaccination. Therefore, the search for new methods of suppressing the reproduction of the virus is an important problem in the poultry industry. Avian infectious bronchitis virus is an acute highly contagious viral disease, mainly of chickens, accompanied by damage to the respiratory organs in young animals and loss of productivity in adult laying hens. The exploitation of medicinal plants is an important topic for both pharmaceuticals and medicine in general. Many viruses have developed resistance to existing drugs, so it makes sense to study plants that contain biologically active compounds that are able to suppress viral activity. The purpose of the research is to show the possibility of using extracts of some plants to inhibit the reproduction of the avian infectious bronchitis virus. It has been shown that at a dose of 3 to 4 μg/ml, plant extracts of *Alnus incana*, *Hedysarum neglectum*, *Polygonum aviculare*, *Alhagi pseudalhagi* are able to suppress 50% of the reproductive activity of the avian infectious bronchitis virus.

**Key words:** *Alnus incana*, *Hedysarum neglectum*, *Polygonum aviculare*, *Alhagi pseudalhagi*, poultry industry, antiviral activity, avian infectious bronchitis virus.

**Introduction.** Poultry farming is one of the fastest growing and most dynamic sectors of the global agro-industrial complex. Development of poultry farming is economically conditioned, socially beneficial and the most prospective direction in the provision of food security of the Republic of Kazakhstan. Many regions of Kazakhstan have favourable natural conditions for the development of poultry farming. Like any technological production poultry farming has inherent disadvantages associated with keeping a large number of poultry per unit area and reducing dietary diversity, leading to the development of poultry stress and increasing the possibility of infectious diseases, as well as their rapid spread [1].

Infectious diseases of poultry with signs of respiratory tract are an urgent problem for modern industrial poultry farming [2]. Among the various etiologies of such diseases, infectious bronchitis of chickens is one of the most important diseases of the industrial poultry industry, as it can cause a decrease in the egg production of adult birds, leading to huge economic losses [3].

Vaccine prophylaxis is the main control of the disease, but the introduction of infections from different regions (foreign feed and fertilised eggs) often makes it ineffective [4]. The virus spreads worldwide, causing enormous economic losses to the poultry industry through reduced feed conversion and weight gain as well as re...
egg production and poor egg quality in laying hens, and for some strains of the virus, which are nephropathogenic, there can be extensive mortality [5].

Infectious bronchitis virus presents a unique challenge in controlling it in commercial chickens [6]. Genomic diversity and the ability of IBV to change rapidly have resulted in different serotypes of the virus that offer no cross-protection (Cavanagh and Gelb, 2008). Consequently, attenuated live vaccines used to control the disease must be tailored specifically to the IBV serotype present in the herd. The situation is aggravated by the presence of serologically different strains for which no vaccine is available [7].

Thus, the search for new methods of controlling this infection is a very topical issue in veterinary medicine. One such method is to diversify the diet of poultry in order to increase the immune status of the animals and reduce the exposure of poultry to the virus. A lot of plants are able to suppress viral replication without affecting the host physiology or with only a few side effects [8]. The molecular pathways associated with the antiviral action of plant extracts can vary from virus to virus. Over the past few decades, natural phytochemicals have been tested for their antiviral properties. However, as viral infections are becoming a significant threat to humans, further research is still needed to gain effective knowledge of viral infections. In addition, medicinal plants are increasingly being proposed as suitable alternative sources of antiviral agents. [9], [10]

Extracts of Houttuynia cordata, Sambucus nigra, Mentha piperita, Tymus vulgaris, Desmodium canadense, Astragalus, Glycyrrhiza radix and Forsythia suspensa are known to be effective against IBD in vitro [11], [12].

Our studies examined the effect of extracts of Alnus incana, Hedysarum neglectum, Polygonum aviculare, Alhagi pseudoalhagi on the ability to suppress the reproduction of avian infectious bronchitis virus.

Alnus incana is widely used in folk medicine as an astringent, antitumor agent. It is also used to treat colds, rheumatism, and joint diseases. Alder is an official medicinal plant. Stems of alder are recommended as a hardening, astringent. In addition, scientific medicine discovered its wound healing, styptic, antitumor, antibacterial and antiviral properties [13]. The red root has anti-inflammatory, diuretic, analgesic, hematopoietic, hemostatic, expectorant effects, stimulates the central nervous system, improves immunity and has a strong antiviral effect [14]. Using Hedysarum species, antiviral drugs are developed, which have activity against DNA-containing viruses, immunostimulating properties, and have a bacteriostatic effect against gram-positive and gram-negative bacteria [15], [16]. Polygonum aviculare is known for its antifungal, antibacterial, antioxidant, anti-cancer, anti-diabetic, neuropharmacological and antiviral features [17].


Infectious bronchitis virus - strain H120, vaccine strain originally isolated in The Netherland in 1956 and passaged in chicken embryo for 120 generation.

Virus was grown in the allantois cavity of 11-day-old chicken embryos (CE) for 96 h at 37°C. Infectious titer of virus was determined on chicken embryos by limiting dilution method. The presence of the virus was determined by PCR. Virus infectivity titer was calculated according to the method of Reed and Mench [18], [19] and expressed in lg EID50/ml. The virus titer in allantois fluid was 10^5 EID100/ml.

The plant preparation was obtained by water-ethanol extraction of plant raw materials. Plant tissues were crushed to a particle size of 2-3 mm. To remove lipids, the crushed raw material was treated twice for four hours with 5-fold volume of acetic acid ethyl ester. Extraction was carried out with a 5-fold volume of 80% ethanol for four hours. The obtained extract was filtered and dried at a temperature not higher than 56°C.

Preparative chromatography of ethanol extract of plant preparations.

The chromatography was performed on an Agilent 1200 HPLC system with a Supelco® HS-C18, 5 µm, 4.6 x 250 mm analytical column.

Sample preparation - a 0.1% aqueous solution of dried ethanol extract of 4 plants (extract from 2012, bulk) were prepared.

Method of analysis:

The mobile phase consisted of two components: distilled water - eluent A and acetonitrile - eluent B. Chromatography was performed for 40 min at a gradient change in the concentration of eluent B of 0-95%.

- Elution rate 1 ml/min.
Sample volume applied 50 µl
- The concentration of the applied sample is 0.1% aqueous solution.
- The chromatographic process was monitored spectrophotometrically at 210 nm and 254 nm.

Determination of the virus inhibitory properties of the compounds studied.

Virus inhibitory properties of the compounds were studied in experiments with avian infectious bronchitis virus on chicken embryos. The study of antiviral activity was carried out by quantitative real-time PCR of viral nucleic acid from allantoic fluid.

RNA isolation.

RNA was isolated by a standard technique using a reagent kit PureLink Viral DNA/ RNA kit (invitrogen), according to the kit manufacturer's recommendations

Obtaining cDNA.

For obtaining cDNA the following components were added in a test tube in the following order per sample: DEPC treated water - 2 µl, Random Hexamer primer - 0.5 µl, (0.2 µg/ µl), 10 mM dNTP - 0.5 µl, RNA template - 4 µl. This mixture was heated at 65°C for 5 minutes, cooled on ice cubes, then added 2 µl of 5X Reverse Transcription buffer 1 µl (50 Units Reverse Transcriptase).

The cDNA synthesis was performed under the following conditions:
- 25°C – 10 min
- 50°C – 30 min
- 85°C – 5 min
- 4°C – end

The polymerase chain reaction (PCR) was performed in 20 µl of reaction mixture containing:
10 µl of Maxima SYBR Green qPCR Master Mix (2X) (Thermo Scientific), 0.5 µl of 10 µl of PanCoV primers targeting a 452 bp fragment of the polymerase gene, (Forward GGG DTG GGA YTA YCC HAA RTG YGA) and (Reverse TAR CAV ACA ACI SYR TCR TCA ) [20], 2 µl cDNA matrix, 0.04 µl 5 µM ROX and 6, 96 µl Nuclease free water.

The PCR reaction was performed on a “PicoReal” Real-Time PCR instrument under the following conditions:
- 95°C – 5 min;
- 35 cycles: 94°C – 1 min, 50°C – 1 min, 72°C – 1 min

Research results. To study the ability of plant extracts to suppress the reproduction of avian infectious bronchitis virus, alcoholic extracts of plants were obtained. The qualitative composition of the preparations obtained was studied by the method HPLC and it was established that the preparations are complex mixtures containing compounds with different hydrophobicity. There 4 chromatography results are given for each plant extract.

![Alnus incana HPLC](image)

Figure 1 – HPLC of *Alnus incana*
According to the results of chromatography (figures 1, 2, 3, 4), absorption peaks were recorded at 254 nm. At this wavelength there is an absorption mainly of the double bonds of the phenolic rings of compounds that are present in given plant extracts. Therefore, since the peaks at this wavelength are
observed, there are compounds of phenolic nature in these extracts which may have antiviral activity. Consequently, the extracts, *Alnus incana*, *Hedysarum neglectum*, *Polygonum aviculare*, and *Alhagi pseudalhagi* could theoretically have an antiviral effect.

![Figure 5 – Effect of some medicinal plants extracts on reproduction infectious bronchitis virus](image)

Abscissa axis represents the dose of extract (µg / ml) inhibiting 50% reproduction of infectious bronchitis virus, strain H120, while ordinate axis demonstrates four different plant preparations, namely *Alnus incana*, *Hedysarum neglectum*, *Polygonum aviculare*, and *Alhagi pseudalhagi*.

As a result, the study of the effect of plant extracts *Alnus incana*, *Hedysarum neglectum*, *Polygonum aviculare* and *Alhagi pseudalhagi* for the reproduction of avian infectious bronchitis virus, strain H120.

It was shown that the selected drugs at a dose are able to suppress 50% of the reproduction of the virus in the embryo (Figure 5), which indicates a pronounced antiviral effect of these preparations on the reproduction of this coronavirus. *Hedysarum neglectum* and *Alhagi pseudalhagi* suppress the virus at a dose of 3.4-3.3 µg/ml, respectively, while *Alnus incana* and *Polygonum aviculare* suppress the virus at 3.6-3.5 µg/ml, respectively. *Alhagi pseudalhagi* inhibits 50% of virus reproduction at the lowest dosage (3.3 µg/ml).

**Conclusion.** To sum up, an infectious bronchitis virus is estimated to be one of the most significant threats to the poultry industry, leading to reduced feed conversion and weight gain as well as wastage in processing plants. Losses are also associated not only with reduced egg production and poor egg quality in laying hens, and for some strains of the virus, which are nephropathogenic, there can be extensive mortality. One of the current methods of controlling this infection is to diversify the diet of poultry, namely using medicinal plants such as *Alnus incana*, *Hedysarum neglectum*, *Polygonum aviculare*, and *Alhagi pseudalhagi* as nutritional supplements to improve the immune status of animals and reduce the impact of the virus on poultry.

Consequently, preparation of plant extracts (µg / ml) inhibiting 50% reproduction of infectious bronchitis virus strain H120 was carried out and tested on chicken embryos. Following the results, the plant extracts of *Alnus incana*, *Hedysarum neglectum*, *Polygonum aviculare*, and *Alhagi pseudalhagi* are able to inhibit 50% of the reproduction of the virus in the embryo, at a certain range of dose starting from 3.6 -3.3 µg/ml. Thus, a certain dosage of plant extracts with antiviral activity inhibiting 50% of the virus reproduction has been identified, which is of interest for their further study with a view to making poultry feed on their basis.

**REFERENCES**


шаруашылығының маңызды мәселесі болып табылады. Құстардың жұқпалы бронхит вирусы – жас жануарлардың тынысы алу мүшеңінің закымдануынан, соны ересек жұмырткалайтын тауықтардың өнімділігінің тамырысымен жүретін, негізінен тауықтардың жедел, оте жұқпалы вирустық ауруы. Вирустардың белсенділігінің басуға қабілетті препараттарды іздеу оларды дәрілік осімдіктер арасында іздеуге мүмкіндік береді. Дәрілік осімдіктерді пайдалану фармацевтика ушін де, жалпы медицина ушін де құстардың тақырып болып табылыды. Қондеген вирустар бар препараттарға тәзімділікті дамыту, сондықтан вирустық белсенділігін басуға қабілетті препараттарды іздеу өсімдікті басуға қабілетті. Құстардың инфекциялық бронхит вирусының көбеюін басу үшін кейбір Alnus incana, Hedysarum neglectum, Polygonum aviculare және Alhagi pseudalhagi вирусқа қарсы препараттар жасау үшін перспективалы өсімдік болып табылады. Зерттеудің мақсаты құстардың инфекциялық бронхит вирусының қобеңі іздеу үшін кейбір Alnus incana, Hedysarum neglectum, Polygonum aviculare және Alhagi pseudalhagi осімдіктердің сығындыларын пайдалану мүмкіндігін көрсету болды. 3-тен 4 мкг/мл-ге дейінгі дозада осімдік сығындылары құстардың инфекциялық бронхит вирусының поративті белсенділігін 50%-га басуға қабілетті екендігі көрсетілген.

РЕЗЮМЕ

Разнообразие вариантов вируса инфекционного бронхита птиц создает определенные трудности при вакцинации. Поэтому поиск новых методов подавления рекомбинации вируса является важной проблемой в птицеводстве. Вирус инфекционного бронхита птиц это острое высококонтагиозное вирусное заболевание, преимущественно цыплят, сопровождающееся поражением органов дыхания у молодняка и потерей продуктивности у взрослых кур-несушек. Поиск лекарственных средств, которые способны подавлять активность вирусов дает возможность искать таковые среди лекарственных растений. Использование лекарственных растений является важной темой как для фармацевтики, так и для медицины в целом. Многие вирусы выработали устойчивость к существующим препаратам, поэтому имеет смысл изучать растения, которые содержат биологически активные соединения, способные подавлять вирусную активность. Благодаря большому количеству активных соединений, Alnus incana, Hedysarum neglectum, Polygonum aviculare и Alhagi pseudalhagi являются перспективными растениями и для создания противовирусных препаратов. Цель исследования - показать возможность использования экстрактов некоторых растений для подавления рекомбинации вируса инфекционного бронхита птиц. Показано, что в дозе от 3 до 4 мкг/мл растительные экстракты серой ольхи, красного корня, горца птичьего и верблюжьей колючки способны на 50 % подавлять рекомбинационную активность вируса инфекционного бронхита птиц.