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PHENOLIC ACIDS OF PLANT ORIGIN AS A PROMISING SOURCE FOR THE DEVELOPMENT OF ANTIVIRAL AGENTS

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Actuality. Seasonal outbreaks of respiratory infections leading to severe complications and high mortality are characteristic of the influenza virus type A. ,Various etiotropic antiviral agents have been developed since the middle of the last century against influenza epidemics and pandemics. The rapid adaptation of the influenza virus and the emergence of drug-resistant strains are the serious problems in the influenza infection treatment. Therefore, the effectiveness of newly developed drugs is determined based on an assessment of their interaction with drug-resistant influenza virus strains. Phenolic acids of plant origin are promising compounds for the development of antiviral agents with a wide spectrum of biological activity and are less susceptible to the emergence of resistant to them strains.

The aim of this work was a comparative study of the antiviral activity of four phenolic acids: Gallic, Syringic, Vanillic and Protocatechuic against influenza A virus in in ovo experiments.

Material and methods. In the present work on the model of two drug-resistant strains of the influenza A virus a comparative study of the antiviral activity of four plant origin phenolic acids: Gallic, Syringic, Vanillic and Protocatechuic is carried out.

Results and discussion. It was shown that Gallic acid is able to inhibit the reproduction of influenza virus A/H5N3 by 50% at a concentration of 1.35 μg/mL; and at a concentration of 0.1 μg/mL is able to inhibit the 50% reproduction of influenza virus A/H3N2, which is comparable with the activity of last-generation anti-influenza drug Tamiflu.

Conclusion. It was shown that for the pronounced antiviral activity phenolic acid must have at least two hydroxyl groups in molecule. The replacement of hydroxyl groups to methoxy leads to an almost complete loss of antiviral properties by phenolic acid. The gallic acid is a potential candidate for the development of promising agents for the treatment of influenza A virus.

Keywords: influenza, Gallic acid, Protocatechuic acid, antiviral activity.

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Т Ұ Ж Ы Р Ы М

ВИРУСҚА ҚАРСЫ ҚҰРАЛДАРДЫ ӘЗІРЛЕУДЕ ПЕРСПЕКТИВАЛЫ КӨЗ РЕТІНДЕГІ ӨСІМДІК ТЕКТЕС ФЕНОЛ ҚЫШҚЫЛДАРЫ

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«Микробиология және вирусология ғылыми өндірістік орталығы» ЖШС, Алматы қ., Қазақстан Республикасы

Жұмыстың өзектілігі. Ауыр асқынуларға және өлімнің жоғары деңгейіне әкелетін тыныс жолдары инфекцияларының маусымдық аурулары А типті тұмау вирусына тән. Тұмаудың індеттері мен пандемияларымен күресу үшін өткен ғасырдың ортасынан бастап вирусқа қарсы әртүрлі этиотропты дәрілер әзірленуде. Тұмау инфекциясын дәрі-дәрмекпен емдеу кезінде тұмау вирусының тез бейімделуі және дәріге төзімді штаммдардың пайда болуы маңызды проблема болып табылады. Сондықтан, жаңадан әзірленетін дәрілік заттардың тиімділігі олардың тұмау вирусының дәріге төзімді штаммдарымен өзара әрекеттесуін бағалау негізінде анықталады. Өсімдік тектес фенол қышқылдары биологиялық белсенділіктің кең спектрі бар вирусқа қарсы препараттарды жасау үшін перспективалы қосылыстар болып табылады және оларға резистентті штамдардың пайда болуына аз ұшырайды.

Зерттеудің мақсаты. *In ovo* эксперименттерінде төрт фенолды қышқылдардың вирусқа

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қарсы белсенділігін салыстырмалы зерттеу: сиреналық, галлдық, ванилин және протокатехты А тұмауы вирусына қарсы.

Материал және әдістері. Осы жұмыста А тұмауы вирусының екі дәріге төзімді штаммдарының үлгісінде төрт өсімдік фенол қышқылдарының: галл, сирен, ванилин және протокатехтың вирусқа қарсы белсенділігіне салыстырмалы зерттеу жүргізілді.

Нәтижелері және талқылауы. Галл қышқылы 1,35 мкг/мл концентрациясындағы A/H5N3 тұмауы вирусының репродукциясын 50% - ға, ал концентрациядағы 0,1 мкг/мл A/H3N2 тұмауы вирусының репродукциясын басуға қабілетті екендігі анықталды, бұл Тамифлудың соңғы буындағы гриппозға қарсы препаратының белсенділігімен салыстыруға болады.

Қорытынды. Белгіленген вирусқа қарсы белсенділіктің көрінісі үшін фенол қышқылының құрамында кемінде екі гидрокси тобы болуы керек екендігі көрсетілді. Гидрокси топтарын метокси тобына ауыстыру олардың вирусқа қарсы қасиеттерін фенол қышқылымен толықтай жоғалтуға әкеледі. Галл қышқылы - А тұмауын вирусын емдеуге арналған перспективті препараттарды әзірлеу үшін ықтимал кандидат.

Негізгі сөздер: тұмау, галл қышқылы, протокатех қышқылы, вирусқа қарсы белсенділік.

Р Е З Ю М Е

ФЕНОЛЬНЫЕ КИСЛОТЫ РАСТИТЕЛЬНОГО ПРОИСХОЖДЕНИЯ КАК ПЕРСПЕКТИВНЫЙ ИСТОЧНИК ДЛЯ РАЗРАБОТКИ ПРОТИВОВИРУСНЫХ СРЕДСТВ

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Сезонные вспышки респираторных инфекций, приводящих к тяжелым осложнениям и высокой смертности, характерны именно для вируса гриппа типа А. Для борьбы с эпидемиями и пандемиями гриппа с середины прошлого столетия разрабатываются различные этиотропные противовирусные средства. Серьезной проблемой при лекарственной терапии гриппозной инфекции является быстрая адаптация вируса гриппа и появление лекарственно-устойчивых штаммов. Поэтому эффективность вновь разрабатываемых лекарственных средств определяется на основе оценки их взаимодействия с лекарственно-устойчивыми штаммами вируса гриппа. Фенольные кислоты растительного происхождения являются перспективными соединениями для создания противовирусных препаратов с широким спектром биологической активности и менее подвержены к появлению резистентных к ним штаммов.

Цель работы. Сравнительное изучение противовирусной активности четырех фенольных кислот: сиреневой, галловой, ванилиновой и протокатеховой против вируса гриппа А в экспериментах *in ovo*.

Материал и методы. В настоящей работе на модели двух лекарственно-устойчивых штаммов вируса гриппа А проведено сравнительное изучение противовирусной активности четырех растительных фенольных кислот: галловой, сиреновой, ванилиновой и протокатеховой.

Результаты и обсуждение. Установлено, что галловая кислота в концентрации 1,35 мкг/мл способна на 50% подавлять репродукцию вируса гриппа A/H5N3, а в концентрации 0,1 мкг/мл - репродукцию вируса гриппа A/H3N2, что сопоставимо с активностью противогриппозного препарата последнего поколения Тамифлю.

Выводы. Показано, что для проявления выраженной противовирусной активности фенольная кислота в своем составе должна иметь не менее двух гидрокси-групп. Замена гидрокси-групп на метокси-группу приводит к практически полной потере фенольной кислотой своих противовирусных свойств. Галловая кислота является потенциальным кандидатом в разработке перспективных лекарственных средств для терапии вируса гриппа А.

Ключевые слова: грипп, галловая кислота, протокатеховая кислота, противовирусная активность.

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To date there are about 2000 different by antigenic spectrum variants of influenza virus [1]. All age and social categories of people are susceptible to this virus. Every 2-3 years a change in the antigenic determinants of influenza A virus circulating in a certain area occurs which leads to epidemics or serious outbreaks of the disease [2]. The main reasons for the rapid spread of the influenza virus among the population are high contagiousness of the pathogen, airborne transmission and high population density in modern society.

Vaccination and etiotropic antiviral drugs are used for the prevention and treatment of influenza. The effectiveness of vaccination is directly related to the timing of its implemen-

tation before the epidemic starting. The composition of the influenza vaccine also is very important, specifically the correspondence of the vaccine strain to the epidemic strain. The effectiveness of drug therapy is determined by the sensitivity of the epidemic virus to a specific drug. In this case the mechanisms of action of the antiviral drug may vary significantly. Thus the adamantane-type drugs (Rimantadine) prevent the cleavage of the surface viral antigen hemagglutinin and as a result the fusion of the viral membrane with the lysosomal vacuole due to the blocking of the viral membrane M2 protein, which makes it impossible for the virus enter to the host-cell [3]. There is other mechanism of action for inhibitors of the viral enzyme neuraminidase (oseltamivir, zanamivir, etc.) which prevent the process of viral particles budding from the host-cell by interacting with the active center of the enzyme [4].

A significant drawback of commercial synthetic anti-influenza drugs is the emergence of resistant influenza virus strains. At the same time neuraminidase inhibitors are more sustainable to the appearance of resistance in the influenza virus epidemic strains, at the moment; but have less clinical efficacy and higher cost than adamantane-type drugs which makes them less accessible for the use by a wide range of population [5, 6].

For these reasons the search and further development of drugs for the prevention and treatment of influenza for the introduction into the clinic remains relevant today. Since the 80s of the last century there has been increased research interest to antiviral drugs of plant origin [7, 8]. This interest is due to the low toxicity of plant preparations, their polyvalences, and mild action which allows the use of these drugs for a long time without side effects for health.

Phenolic compounds of plant origin have a wide range of biological activity including antiviral [9, 10]. They are found in almost all higher plants. Phenolic acids found in various plants, fruits and vegetables have such biological properties as antioxidant, antiviral, anticancer, antimicrobial, anti-inflammatory [10, 11, 12].

The aim of this work was a comparative study of the antiviral activity of four phenolic acids: Gallic, Syringic, Vanillic and Protocatechuic against influenza A virus in *in ovo* experiments.

MATERIAL AND METHODS

Avian influenza virus strain A/Tern/South Africa/1/1961 (H5N3) resistant to commercial antiviral drugs (Rimantadine, Tamiflu) and human epidemic influenza virus strain A/Almaty/8/98 (H3N2) were used.

Viruses were grown in the allantoic cavity of 10 days old chick embryos (36 h., 37°C). The virus titre was 10^7-10^9 EID50/mL in the allantoic fluid.

The hemagglutination activity of viruses was determined according to the standard method [13] using 0.75% suspension of chicken red blood cells.

As objects of study purified plant origin phenolic acids were used: Gallic, Syringic, Vanillic and Protocatechuic with different amounts of hydroxyl and methoxy groups. The structural formula of the studied phenolic acids is presented in Figure 1.

The study of the specific antiviral activity of phenolic acids of plant origin was carried out in accordance with the methodological recommendations of the "Guidelines for preclinical studies of drugs" [14, 15]. The main criterion at the study of the specific antiviral effect of the compounds is the chemotherapeutic index (CTI), which is determined by the ratio of the average toxic concentration of the substance (TC_{50}) to the average effective virus-inhibiting concentration (EC_{50}).

Rimantadine®, Olanpharm, Latvia (alpha-methyltricyclo [3.3.1.1/.7] decan-1-methanamine as hydrochloride, CAS 13392-28-4) and Tamiflu®, Hoffmann-La Roche, Switzerland (3R,4R,5S)-4-acetylamino-5-amino-3-(1-ethylpropoxy)-1-cyclohexene-1-carboxylic acid ethyl ester, phosphate, INN oseltamivir, CAS 196618-13- 0) [16, 17] were used as reference drugs.

For mathematical processing of the results standard methods for finding the mean values and their average errors were used [18].

RESULTS AND DISCUSSION

Study of the antiviral activity of 4 phenolic acids of plant origin (Gallic, Syringic, Vanillic and Protocatechuic acids) against human and avian influenza viruses was carried out.

A study of the ability of compounds to inhibit the reproduction of influenza virus was carried out in the range of doses from 0.1 μg/mL to 2.5 μg/mL.

It was shown that in a studied dose range the structure of phenolic acid significantly affects the ability to suppress the reproduction of the influenza virus (Figures 2, 3). In the studied dose range only gallic acid was able to effectively suppress the reproduction of the influenza virus (inhibit more than 50% of 100 infectious doses of the virus) regardless of its antigenic structure. An interesting fact is that protocatechuic acid which effectively suppresses the reproduction of the A/H5N3 influenza virus turned out to be ineffective against the A/H3N2 influenza virus.

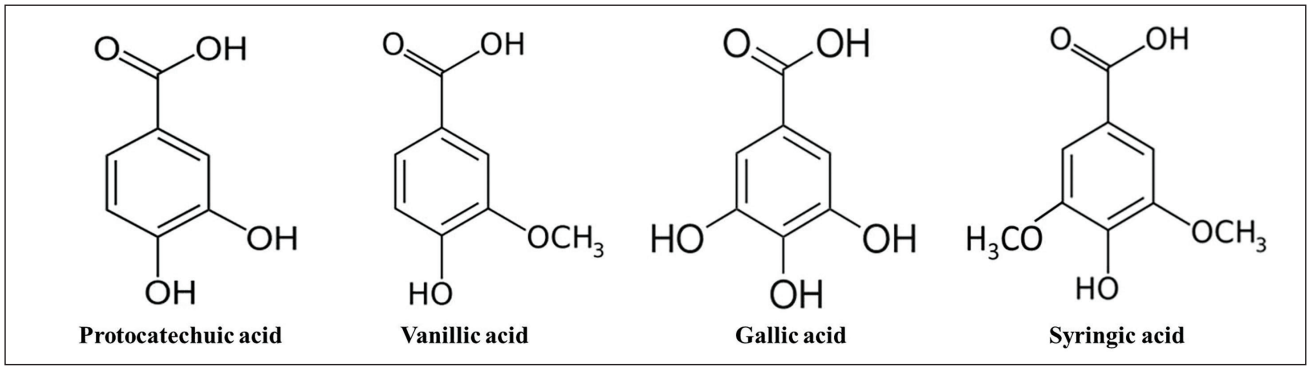

Figure 1 – The structure of the studied phenolic acids of plant origin

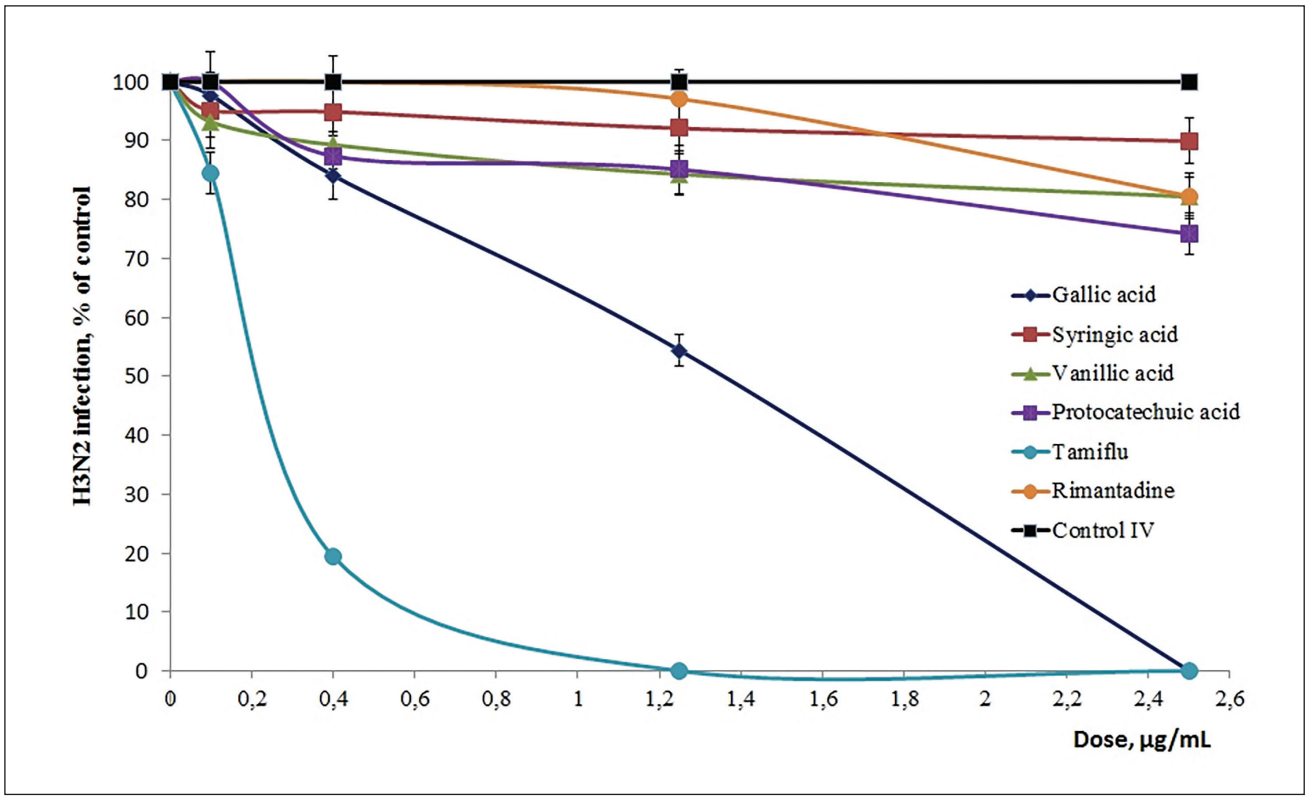

Figure 2 - The dose-dependent effect of the virus-inhibiting activity of phenolic acids on the model of human influenza virus strain A/Almaty/8/98 (H3N2). Control IV – influenza virus

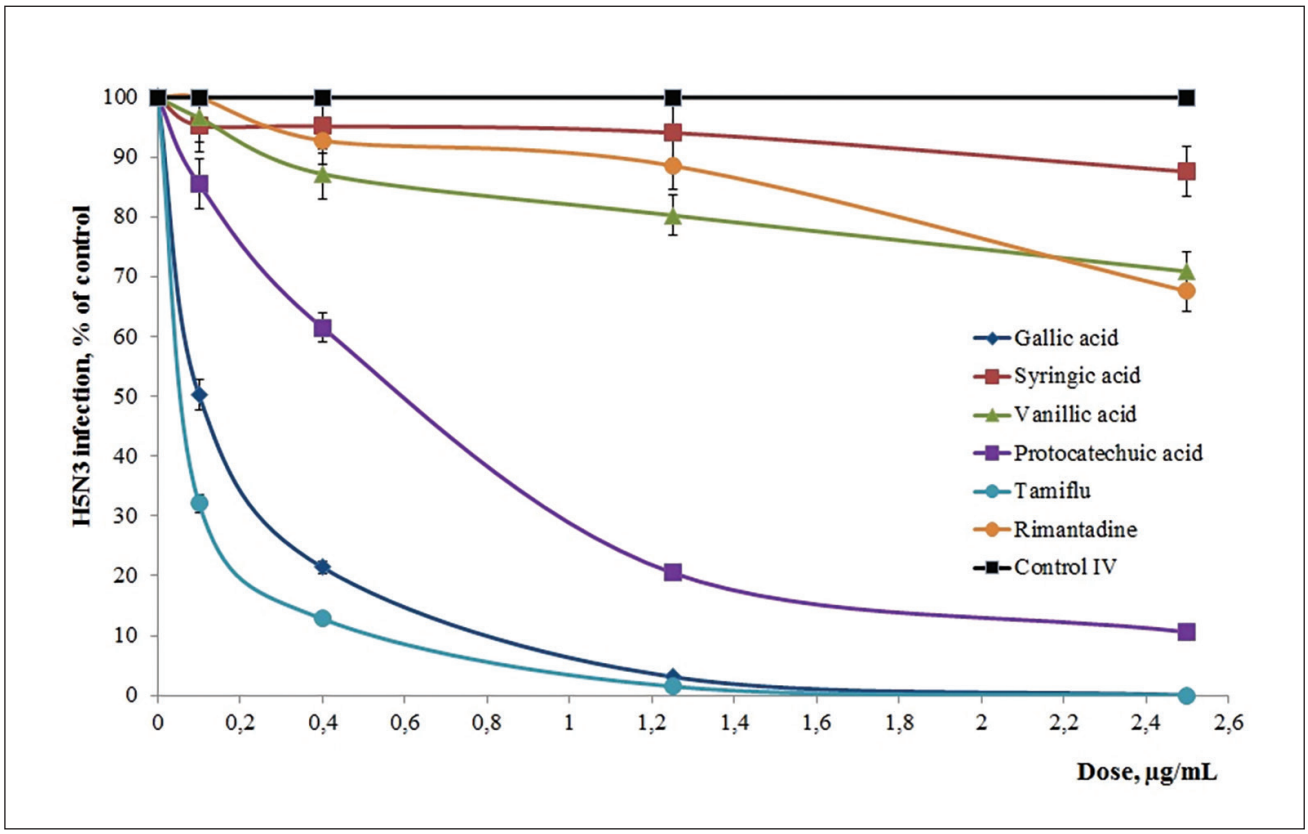

Figure 3 - The dose-dependent effect of the virus-inhibiting activity of phenolic acids on the model of avian influenza virus strain A/Tern/South Africa/1/1961 (H5N3). Control IV – influenza virus

Most antiviral activity research including prophylactic and therapeutic are carried out in *in vitro* experiments believing that these data can be extrapolated to live models *in vivo*. 10-12 days old chicken embryos are alternative to cell culture and animal model for influenza virus propagating and antiviral activity investigation. The main advantage of chicken embryos is that the virus propagated in the germinal membranes, secreted into the allantoic and amniotic fluid where it accumulates in large quantities. The use of chicken embryos led to a genuine revolution in the study of viruses, which was continued by the introduction of tissue culture into the virological technique. At the same time the model of chicken embryos for propagation and investigation of susceptible viruses has retained its significance today. Despite its shortcomings the method is relatively simple, convenient, and cheap and is widely used in virological studies [19, 20].

In our research a comparative study of the anti-influenza activity of four phenolic acids differing in amounts of hydroxyl and methoxy groups was carried out. It was shown that the severity of antiviral activity is directly related to the number of hydroxyl groups in the phenolic acid molecule. The greatest antiviral activity in *in ovo* experiments on both strains of model influenza A viruses was shown by gallic acid, which had 3 hydroxyl groups in its molecule at C3, C4 and C5 positions. The anti-influenza activity of gallic acid in relation to the A/H5N3 influenza virus strain resistant to commercial anti-influenza drugs was comparable to the activity of the commercial antiviral drug Tamiflu, the most effective anti-influenza drug known to date (table 1). Significant antiviral activity of gallic acid was also shown against the epidemic strain of human influenza virus A/H3N2.

Table 1 – **EC_{50} of tested compounds on the model influenza A virus strains**

Compound	Antiviral activity, μg/mL	
	H3N2	H5N3
Gallic acid	1.35±0.055	0.1±0.004
Protocatechuic acid	Na*	0.59±0.029
Tamiflu	0.22±0.01	0.05±0.002
*Na – Concentration not achieved		

Protocatechuic acid having only 2 hydroxyl groups (C3, C4) showed less pronounced antiviral activity than gallic acid on the model influenza virus strain A/H5N3. In the studied dose range for protocatechuic acid it was not possible to determine the EC50 on the epidemic strain of influenza virus A/H3N2.

Syringic acid in the studied dose range was able to suppress only up to 12% of reproduction of the influenza virus A/H5N3 and A/H3N2. Substitution of two hydroxyl groups (C3 and C5) by methoxy groups in the molecule of syringic acid apparently leads to almost complete loss of antiviral properties by this substance.

Vanillic acid which differs from protocatechuic acid by replacing C3 - hydroxyl by a methoxy group in the studied dose range was able to suppress the reproduction of model influenza A viruses by no more than 29%.

CONCLUSION

It was shown that for the pronounced antiviral activity phenolic acid must have at least two hydroxyl groups in molecule. The replacement of hydroxyl groups to methoxy leads to an almost complete loss of antiviral properties by phenolic acid. In model of *in ovo* experiments on Rimantadine-resistant strains of influenza A viruses showed that gallic acid is able to suppress 50% of the reproduction of the epidemic influenza virus A/H3N2 at a dose of 1.35 μg/mL, and the influenza virus A/H5N3 at a dose of 0.1 μg/mL. The dose of protocatechuic acid capable of suppressing 50% of the A/H5N3 influenza virus activity is 0.59 μg/mL. Thus, gallic acid is a potential candidate for the development of promising agents for the treatment of influenza A virus. However, additional investigations are needed to study the mechanism of the antiviral effect of this substance.

Research transparency

Research did not have a sponsorship. The authors are absolutely responsible for presenting the release script for publication.

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Declaration about financial and other relations

The authors did not get the honorary for the article.

Authors' contributions

Aizhan Turmagambetova - conceived and directed the study, performed the experiments and prepared the report that accompany the data, created figures and tables, analyzed data and prepared the manuscript, provided statistical expertise in data analysis.

Andrey Bogoyavlenskiy - conceived and directed the study, performed the experiments and prepared the report that accompany the data, provided statistical expertise in data analysis.

Pavel Alexyuk and Madina Alexyuk - performed the experiments and prepared the report that accompany the data.

Vladimir Berezin - conceived and directed the study, provided factual review, inputted conceptual ideas and edited the manuscript. All authors read and approved the final version of the manuscript.

Conflict of interest

The authors declare no conflict of interest.

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