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Review Article

Study of the Antiviral Activity of Propolis Phenolic Fraction

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Abstract

The antiviral activity of the propolis phenolic fraction of (PPF) against influenza virus, adenovirus and coronavirus and vesicular stomatitis virus was studied. As a result of the performed study the maximum tolerated concentration (MTC) of cell culture of human or animal origin was determined, which was 1.0% for the lot of PPF № 1 and 0.5% for the lot of PPF № 2. An aqueous solution of PPF (lot № 1) decreased coronavirus hemagglutinin titer by 4 - 8 times, thus indicating the prospects of using the drug against coronavirus infections.

Keywords: Propolis Phenolic Fraction; Antiviral Activity; Maximum Tolerated Concentration

Currently, all over the world, including in Ukraine, viral infections are one of the most important problems of medical science and health due to the widespread prevalence, high incidence, frequent development of protracted and chronic severe consequences for young children.

Influenza viruses play a special role among a variety of groups of viruses that affect humans. In recent years, these viruses have caused epidemics of varying intensity. According to the World Health Organization, influenza accounts for 70 - 80% of all infectious diseases in all countries around the world. Influenza virus, as the most important viral pathogen, is devoted to numerous works to study its molecular structure, mechanisms of interaction with sensitive cells, etc. the effectiveness of influenza vaccines is one of the most difficult tasks. Until now, a small number of antiviral drugs have been used to treat and prevent influenza. This is largely determined by the peculiarities of the parasitism of viruses that affect the cell genome. In this regard, an important requirement for chemical drugs is the ability to influence them only directly on the virus itself, without damaging the cell itself, in which the virus parasites.

An important group of viruses that are widespread and cause a variety of pathologies are adenoviruses. Their role in respiratory and intestinal diseases, ophthalmology, otolaryngology, as well as the ability of some serotypes to cause cell transformation has been established. The relevance of the development of antiviral drugs in relation to adenoviruses is associated with the lack of effective therapeutic and preventive drugs.

Coronaviruses are a group of viruses whose role in respiratory and intestinal pathology has been established relatively recently. Studying these viruses and finding antiviral drugs in relation to coronaviruses is a priority for Ukraine, as for the first time in the Kharkiv Institute of Microbiology and Immunology. I.I. Mechnikova created a test system to identify them and research is carried out to study coronavirus diseases.

There is also a clear need to look for harmless and effective antiviral drugs for the vesicular stomatitis virus (VVS), which causes damage to the mucous membranes of the mouth, nose and pharynx.

It is known that many chemical compounds with antiviral activity have a side effect in the form of high toxicity, teratogenicity and immunosuppressive properties. In this regard, an important area of scientific research is the detection of antiviral action in drugs derived from raw materials of natural origin.

In the aspect of the foregoing, it is of undoubted interest to create antiviral apipreparations the use of various standardized substances of beekeeping products, in particular - propolis.

Given the widespread prevalence and important role in pathology as a test virus to study the antiviral properties of the

phenolic fraction of propolis (FFP), we have selected influenza viruses, adenoviruses, coronaviruses and VVS.

The data of Yugoslav researchers, pointing for example to the protective effect of propolis drugs for herpes and influenza, are noteworthy. Good preventive effects of propolis drugs were found during the flu epidemic among schoolchildren. To prevent influenza, the drug was used as a mixture of bee honey, 1% propolis solution and 2% of the solution of royal jelly.

Among those receiving the drug, only 6 schoolchildren (9.5%) fell ill, while among those who did not receive the drug - 61 people (38.8%).

At the same time, it is important to note that until now many propolis drugs are used in medicine without proper medical and biological justification, without a detailed and comprehensive study of their mechanism of action. There is no information, in particular, about the effect of propolis drugs on the cells of the body. The need for comprehensive, qualified virological studies using different types of viruses to develop specific and targeted indications for the use of propolis drugs is also important.

In connection with the foregoing, the purpose of our research was to study the antiviral properties of FFP in a number of viruses (influenza, adenoviruses, coronaviruses and vesicular stomatitis virus).

To determine the antiviral activity of different concentrations (0.5%, 1.0% and 2.0%) FFP used *in vitro* (in different cell cultures) and *in vivo* (on developing chicken embryos.

The antiviral activity of the polyphenolic fraction of propolis (FFP) in relation to this group of viruses was determined in the culture of the above cells. The neutralization response was applied in the following way: 0.2 ml of the corresponding virus in the working dose (100 CDC_{50}/0.2 ml) incubated for 10 minutes. At room temperature with 0.2 ml of water solution of the drug and intake in a dose of 0.2 mlin thermostat. Reducing the titers of the virus under the influence of the drug by 2 lq or more in comparison with control was assessed as a manifestation of antiviral activity.

Investigation of the rolling old FSF on non-agglutinating asset

Equal volumes of viruses in the working dose (4 hemagglutining units) and the drug were mixed and after incubation for 30 minutes at room temperature determined the degree of inhibition of hemagglutinizing activity of viruses under the influence of drugs. The controls were: the virus in the working dose without the drug and the corresponding red blood cells - 1 0(I) groups of people for influenza viruses and mice other erythrocytes for the crowns and viruses.

Reducing hemagglutinizing activity of viruses and more times was considered as the effect of the drug on hemagglutinin в 4t and none of the viruses used in the experience.

Study of the antiviral activity of the polyphenol fraction of propolis (FFP)

Study of FFP toxicity in various indigested cell cultures and chicken embryos (concentrations 0.5; 1.0 and 2.0%).

It is known that therapeutic and preventive drugs, as well as various «additives» (stabilizers, preservatives, fillers, etc.) may have a cytotoxic effect.

The method of cellular structures has been increasingly used in recent years to determine the harmlessness of various treatments. This is due to the fact that modern methods used to control safety in laboratory animals are not always sensitive enough to detect toxicity.

In this regard, we conducted a study of the toxicity of F Pon different x cells and the determination of maximum tolerable concentration (MPC)МПК) for each series of the drug. The results of the test of water solutions of FFP on various indigestible cells are presented in the table 1.

As can be seen from the table 1 data, 0.5 and 1,0% concentration of the drug FFP (series No. 1) did not cause cytopathic damage in any of the annexes of the cell cultures. was toxic to the cells. This is the appearance of rounded with grainy cytoplasm cells that were rejected from the surface of the glass.

Series 2 of the drug in the form of 1,0 and 2.0% concentrations caused cell degeneration with impaired integrity of the cell layer already on the 2nd day after the drug's contact with cells.

Thus, Series No. 1 OFP in the form of 0.5 and 1.0% кокоcentering did not have a toxic effect for cells PT, T_2, Hela and Ner-2. The drug in the form of 2.0% concentration caused morphological changes in cells, the degree of which strengthened me by 7 - 8 days and amounted to q.

Series 2 FFP in comparison with series No. 1 was more toxic - in 1.0% concentration the drug caused degenerative changes in all test cells with impaired cell monolayer already on the 2nd day after inhaling.

No.	Cell species	Concentration of the drug as %	Result (toxicity)			
			1 day	2 days	5 days	7-8 days
I	Fri	0,5	–	–	–	±
		1,0	–	–	±	++
		2,0	++	+++	++++	++++
	T_2	0,5	–	–	±	++
		1,0	–	–	++	+++
		2,0	++	+++	++++	++++
	Hela	0,5	–	–	++	++
		1,0	–	–	++	+++
		2,0	++	+++	++++	++++
	Hep-2	0,5	–	–	±	++
		1,0	–	–	+	+++
		2,0	++	+++	++++	++++
II	Fri	0,5	–	–	±	+++
		1,0	++	+++	++++	++++
		2,0	+++	+++	++++	++++
	T_2	0,5	–	–	±	+++
		1,0	++	+++	+++++	++++
		2,0	+++	++++	+++++	++++
	Hela	0,5	–	–	±	++
		1,0	++	+++	++++	++++
		2,0	+++	+++	++++	++++
	Hep-2	0,5	–	–	++	++
		1,0	++	+++	++++	++++
		2,0	+++	+++	++++	++++
Cell control		Fri	–	–	–	+
		T_2	–	–	–	+
		Hela	–	–	+	++
		Hep-2	–	–	+	++

Table 1: Results of the study of the toxicity of FFP on cells of different origins.

For the Series No. 1 FFP, the maximum tolerable concentration was 1.0%, and for series No. 2 - 0.5%.

Study of antiviral activity of different FFP series

The results of the study of the action of F Pon influenza A virus (H_3N_2) in studies on developing daytime chicken 9-10 embryos are presented in the table 2.

And the data presented in the table 2, both series, while administering the drug and the virus, had a low ratio and index of protection of chicken eixions against influenza A virus (H_3N_2).

The study in this regard should be continued in the experiments with the introduction of the drug before infection with the virus

FFP No.	Number of infected embryos	Of these with the virus		Protection Ratio (CP)	% Protection Index (IS)
		Number	%		
Series 1	15	12	84,2	1,1	10
Series 2	15	13	86,7	1,0	0
Control	15	15	100,0		

Table 2: H_3N_2.

(i.e. to study the preventive effect of the drug). In addition, it is advisable to stage studies with other variants of influenza virus - A (H_1N_1) and B.

Are presented in the table, it's not going to be enough 3 H_3N_2. Three of them.

The results of the study are presented in the table 3 indicate that 1.0% of the water solution series No. 1 FFP reduced the titrate of the corona of alphavirus by 4 - 8 times, indicating the prospect of its use for infections caused by the virus.

FFP No.	Titer hemagglutinins before and after the effects of FFP on viruses			
	Flu		Coronarovirus	
	Before	After	Before	After
Series 1	1:64	1:64	1:128	1:161 ± 1:32
Series 2	1:64	1:64	1:128	1:128
Control of the flu virus	1:64	–		
Coronavirus control			1:128	–
Control of red blood cells	no hemagglutination			

Table 3: The really short cut on the hemagglutinin

cut-out Grappa and Coronavirus**.**

In addition, it has also been established that both series of the drug (No. 1 and No. 2) did not reduce the hemagglutinin activity of influenza Virus A (H_3N_2), as the titre of hemagglutinins' before and after exposure to the drug remained unchanged- and composition and l 1:64. That both series of the drug (The study of the antiviral action of FFP in relation to cytopathogenic viruses (adenoviruses and vesicular stomatitis virus) was conducted in the reaction of neutralization on the culture of Ner-2 cells 4.

The data obtained is presented toe in the table 4, series No. 1 FFP (1.0% water solution) shows a delay and of 3.0 lg vesicular stomatitis and vesicular stomatitis virus, which indicates the prospect of using this drug to treat and prevent infections caused by the virus. At the same time, aliments in this direction is desirable to continue with the large number of serotypes of adenoviruses,

FFP No.	Concentration of the drug (%)	Delayed virus reproduction (in Lg) in the neutralization response to the culture of Hep-2 cells	
		Vesicular stomatitis virus	Adenovirus
Series 1	1,0%	3,0	1,0
Series 2	0,5%	0,5	0*
Control of test viruses (100 $CDC_{50}/0_2$ ml		0	0

Table 4: The effect of FFP on adenoviruses and vesicular stomatitis virus.

Note: 0 - No delay of cytopathogenic action of viruses in the working dose.

which cause a variety of clinical manifestations of diseases (respiratory and intestinal infections, eye diseases, etc).

Series 2 FFP had weak antiviral activity (0.5 lg) in relation to vesicular stomatitis virus and did not affect the reproduction of adenoviruses in cell culture.

Thus, in experiments on developing chicken embryos, FFP (series 1 and 2) did not have an inhibitory effect on influenza A virus (H_3N_2) while administering 1.0% of the drug's water solution and virus in the working dose.

In the reaction of inhibition of hemagglutination of cells, series No. 1 FFP gave a decrease of 4 - 8 times hemagglutinizing activity of coronavirus. Series 2 of FFP had no effect on the hemagglutinating activity of coronavirus. In the re-neutralization on the culture of cells Ner-2 series No. 1 FF delayed by № 2 FFP 3.0 lg report the duction of the vesicular stomatitis virus and by 1.0 lg adenovirus.

Study of the effects of ancillary substances on antiviral activity of FFP

At the beginning of our research, the harmlessness of «additives» for cell culture was studied. The results of these shows in the table 5.

No cipher additives	Sobecome Additives in the FSF	Concentration of the drug (%)	Result (toxicity)			
			1 day	2 days	5 days	7-8 days
1	Sugar powder	0,5	–	–	–	+
		1,0	–	–	–	+
		2,0	++	+++	+++	++++
4	Sodium chloride	0,5	–	–	–	+
	Mannit	1,0	–	–	–	+
	Uvl. Water	2,0	++	++	+++	+++
7	Glucose	0,5	–	–	±	+
	Lactose	1,0	–	–	++	+++
	Sah. Powder	2,0	++	+++	+++	++++
	Starch					
	Calcium stearat					
	Uvl. Water					
15a	FSF (Aug. 2)	0,5	+	++	+++	++++
	Sugar	1,0	+++	+++	++++	++++
15v	FSF (Uman)	0,5	–	±	+	+++
	Sugar	1,0	++	+++	+++	++++
		2,0	+++	+++	++++	++++

Table 5: Results of study of FFP's «additives» on Hep-2 cell culture.

It has whether been established (Table 5) that FFP (series No. 2), with added sugar, had a toxic effect on cell culture in 0.5% concentration, and FFP (Uman) with the addition of sax macaw - in 1.0% concentration. They in the cocaine drug will not affect the properties of FFP.

A test of the toxicity of FFP solutions with «additives» was also carried out on developing chicken embryos 6.

As can be seen from the table 6 data, FFP in the form of 1.0% water solution with «additives» was, a non-toxic for chicken embryos. This led to the conclusion that FFP was harmless with «additives» for developing chicken embryos.

The results of the FFP trial with «additives» Nos. 1.4 and 7 for the tested viruses yielded such results, as in the use of the drug without «additives» (perm table 2-4).

Thus, supplements under Nos. 1, 4 and 7 in FFP in the form of 0.5 and 1.0% concentration were harmless to Hep-2 cells. 2.0% concentration of the drug caused cell degeneration on q in a day, the degree of degeneration increased as cells were cultivated. Supplements under Nos. 15a and 15b were toxic to cells. FFP in the form of 1.0% solution was a harmless and for day-to-day chicken embryos. 9 - 10 Supplements under Nos. 1.4 and 7 did not affect the antiviral activity of FFP. Supplements under Nos. 15a and 15b were not used in experiments because of the toxicity of them for cell culture [1-7].

Conclusion

As a result, research was made with the findings:

- Series No. 1 FFP in the form of 1.0% and series No. 2 in the form of 0.5% water solutions when tested on various types of indigested cells of animal and human origin (PT, T_2, Hela and Ner-2) did not have toxic properties. 2.0% water solution FFP is called shaft of degeneration cells.
- In experiments *in vivo*, subjects of latent ration (0.5; 1.0 and 2.0%) FFPs were harmless to developing daytime chicken embryos.

No cipher additives	The drug under study	RESULT (toxicity)	
		Number of living embryos	Number of dead embryos
1	FSF Sugar powder	5	0
4	FSF Sodium chloride Mannit Uvl. Water	5	0
7	FSF Glucose Lactose Sah. Powder Starch Calcium stearat Uvl. Water	4	1
15a	FSF (Aug. 2) Sugar	5	0
15v	FSF (Uman) Sugar	5	0
Embryo control		5	0

Table 6: Effect of 1.0% of FFP solution with «additives» on chicken embryos.

- в 1, supplements to a FFP at 1.4 and 7 in the form of 1.0% concentration were harmless to the culture of indigested cells.
- Inhibition of reproduction of vesicular stomatitis virus on 3.0 lg was established at the same time, while the cell culture was introduced 1.0% of the solution (series No. 1) FF and the working dose of the virus. 0.5% solution did not have antiviral activity.
- PA (H_3N_2). (No. 1 - No 2) - «No. 1- No.
- Water solution FFP (series No. 1) reduced 4 - 8 times the titer of hemagglutinin coronas and the virus, which indicates the prospect of using the drug for crown sand viral infections.

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