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ANTIVIRAL DRUGS BASED ON BIOLOGICALLY ACTIVE SUBSTANCES FROM LARCH WOOD

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The antiviral properties of biologically active extractive substances isolated from the wood of Siberian larch and Gmelin larch have been studied. It was shown that the bioflavonoid dihydroquercetin and the polysaccharide arabinogalactan have a pronounced anti-influenza activity. On their basis, a new complex drug has been developed for the prevention and treatment of influenza and acute respiratory viral infections.

Keywords: *larch wood, arabinogalactan, dihydroquercetin, antiviral activity*

ANTIVIRAL PREPARATIONS BASED ON LARCH WOOD BIOLOGICALLY ACTIVE SUBSTANCES

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Antiviral properties of the extractives isolated from larch wood have been investigated. It has been shown that bioflavonoid dihydroquercetin and natural polysaccharide arabinogalactan possess the expressed anti influenzal activity. A new complex preparation for prophylaxis and treatment of influenza and acute viral respiratory infection has been developed.

Key words: *Larch wood, arabinogalactan, dihydroquercetin, antiviral activity*

Larch wood contains up to 4.5% flavonoids, which are compounds of the same type in terms of chemical structure with a predominant (more than 80%) content of the bioflavonoid dihydroquercetin (DHQ). No less valuable is the natural polysaccharide arabinogalactan (AG), whose content in heartwood reaches 15%. Technology for obtaining these valuable biologically active compounds from Siberian larch and Gmelin larch wood

was developed in the Laboratory of Wood Chemistry of the Irkutsk Institute of Chemistry, Siberian Branch of the Russian Academy of Sciences and has now been brought to the industrial level [1].

Arabinogalactan is a unique soluble natural polysaccharide characterized by a complex of valuable properties,
the most important of which are high biological activity and low toxicity (does not show acute toxicity at a dose of 5 g/kg of body weight and chronic toxicity at a dose of 500 mg/kg per day [5]). Arabinogalactan has an immunomodulatory and prebiotic effect, its use contributes to a significant improvement of the gastrointestinal tract, especially the large intestine [10]. Based on flavonoids isolated from larch wood, the

phytopreparation Diquertin containing more than 90% dihydroquercetin was created [2]. Diquertin, as a drug that helps restore capillary resistance, has anti-inflammatory, anti-edematous effects, has a positive effect on functional state of the liver, recommended for pathogenetic therapy in bronchopulmonary diseases and ischemic disease

heart, unstable angina and supraventricular arrhythmias [3].

On the basis of dihydroquercetin, a number of biologically active food supplements (over 100 items, for example, Siblarin, Kapilar, etc.) have been developed that can significantly improve the quality of nutrition and the immune status of the population living in environmentally unfavorable conditions. To realize the high potential of the biological activity of

the above described natural compounds, their antiviral activity was studied.

MATERIALS AND METHODS

We used samples of dihydroquercetin and arabinogalactan isolated from larch wood according to a previously developed technology [1, 2] and corresponding in terms of quality to the approved regulatory and technical documentation (TU 9354-020-39094141-
07 "Dihydroquercetin"; TU 9363-021-39094141-08 "Fibrolar" (arabinogalactan))

In studies of the antiviral effect of dihydroquercetin and arabinogalactan, outbred white mice of both sexes weighing 10–12 g obtained from the Rappolovo nursery (Leningrad oblast) were used to simulate an experimental lethal influenza infection. Animals were kept on a standard diet

regulated conditions of the vivarium of the Scientific Research Institute of Influenza of the North-Western Branch of the Russian Academy of Medical Sciences. The selection of animals in the experimental groups was carried out by random sampling. Prior to testing, the animals were observed for 2 weeks.

We used influenza A/Aichi/2/68 (H3N2) and B/Lee/40 viruses adapted to white mice.

A sample of the drug was suspended in a minimum amount of tween-80, then the required amount of physiological phosphate buffer was added to a final concentration of 30 mg/mL. The study drug was administered to the animals 2 times a day with a 12-hour interval, 50-100 µl intraperitoneally, starting from 24 hours before infection and ending at 5 days after infection. As a placebo, animals in the control group were injected with physiological phosphate buffer in equal volumes. Remantadine at a dose of 50 mg/kg intraperitoneally was used as a reference drug. The virus was preliminarily titrated on animals and its concentration was determined by the lethality of mice. For the experiment, the virus was administered intranasally to animals under light ether anesthesia at a dose of 0.2 and 5 LD50. Each observation group included 10 mice.

Animals were observed for 15 days; the period during which animal mortality is observed in experimental influenza. Weight was recorded daily and animal mortality in control and experimental groups.

Efficacy testing of the complex drug DQV/AG/AsC (dihydroquercetin/arabinogalactan/ascorbic acid) for the prevention of influenza and other acute respiratory viral infections was carried out in full compliance with the requirements of the World Health Organization and the Committee on Medical Immunobiological Products of the Ministry of Health Russia, in particular, with regard to blind control, i.e. formation of observation groups by random sampling and the use of encrypted drugs. Investigated drug - tablets weighing 1 g. Active ingredient:

dihydroquercetin - 0.015 g; arabinogalactan - 0.260 g; ascorbic acid - 0.015 g. Excipients: sorbitol - 700 mg, calcium stearate - 10 mg.

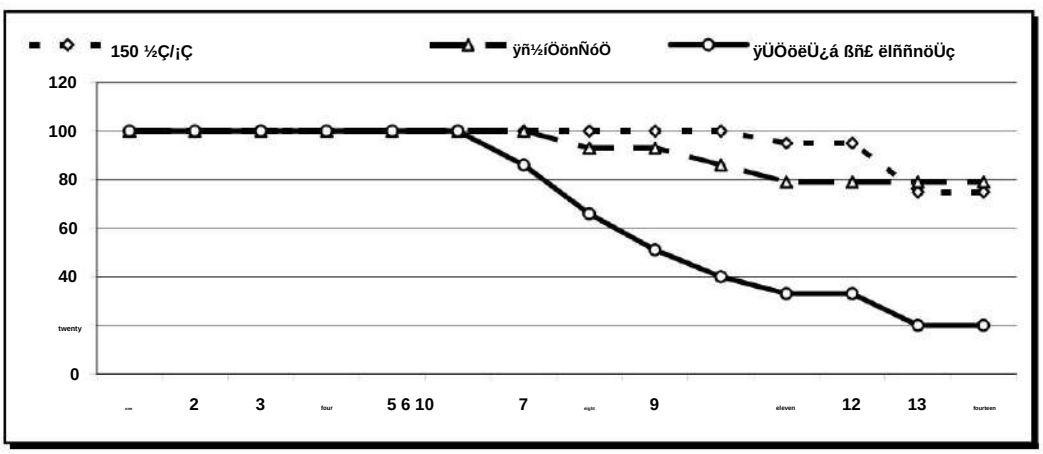
Placebo - tablets with the same content of excipients, but without the active substances.

The test drugs were used in groups with organized meals twice a day, 2 tablets with meals. Under supervision were young men aged 18–25 years in a boarding school team with a close circle round the clock communication in classrooms, bedrooms and with limited external contacts with the population of the city. The study included members of the team who provided informed consent to participate and provided that they met the inclusion and exclusion criteria.

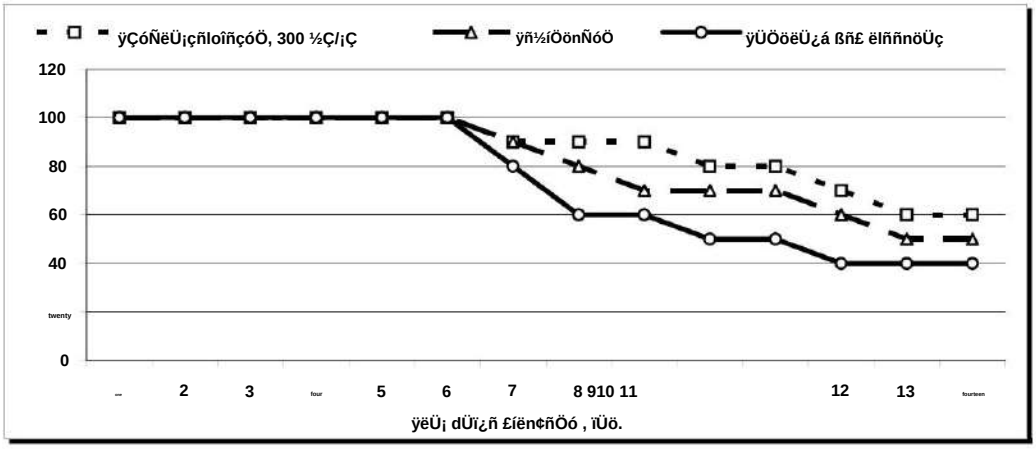
Safety was assessed on the basis of subjective complaints, side effects, and objective clinical examination data.

RESULTS AND DISCUSSION

The study of the antiviral properties of dihydroquercetin in a model of an experimental lethal influenza infection in white mice caused by influenza A and B viruses showed that in the case of influenza A (Fig. 1), the antiviral activity of dihydroquercetin was equal to or even exceeded that of the reference drug rimantadine (protection indices 30–50% when animals are infected with 5 LD50 of the virus, and 87% at a virus dose of 1 LD50 with rimantadine activity of 60–82.5%, depending on the virus dose). In the case of animal infection caused by influenza B virus (Fig. 2), resistant to rimantadine, the indicators



Rice. Fig. 1. Dynamics of animal mortality from a lethal influenza infection caused by the influenza A virus under the conditions of the use of dihydroquercetin at an infectious dose of the virus 5 LD50.



Rice. Fig. 2. Dynamics of animal mortality from a lethal influenza infection caused by the influenza B virus under the conditions of the use of dihydroquercetin at an infecting dose of the virus 1 LD50.

activities of dihydroquercetin were lower, however also outperformed the activity of Reman tadine, which is ineffective in the case of influenza B. Thus, it has been shown that dihydroquercetin exhibits antiviral properties in a model of experimental lethal influenza pneumonia.

monia, regardless of whether it is caused by a virus influenza type A or B [11].

The study of the immunomodulatory properties of arabinogalactan isolated from Siberian larch wood revealed its effectiveness.

in relation to the reactions of cellular and humoral part of the immune response [6]. It significantly increases the level of specific antibodies and the phagocytic activity of macrophages [5].

It is known that some plant polysaccharides have antiviral properties, inhibiting hemagglutination and reproduction of the influenza A virus. It is assumed that the mechanism of action is based on direct adsorption complex formation of the polysaccharide and the virus [4]. When studying the antiviral activity of arabinogalactan, it was shown that it exhibits protective properties in a model of lethal influenza infection when using different doses of the virus. At the same time, the activity of arabinogalactan was 50% at a low infectious dose and 22% at a high one. Considering the low toxicity of this drug, we can talk about the prospects of its use for the prevention and/or therapy of influenza and recommend the further development of arabinogalactan as a means of complex therapy for influenza. The use of preparations based on arabinogalactan opens up new prospects for reliable protection against viral diseases.

Being a soluble dietary fiber, arabinogalactan exhibits prebiotic properties; the ability to selectively stimulate the growth and activity of beneficial intestinal microflora - lacto- and bifidobacteria (probiotics). As a result, it contributes to the preservation

healthy gastrointestinal tract

research institutes. Regular intake of arabinogalactan can normalize immunity not only through direct impact, but also through effects on the intestinal microflora, which, in turn, help the human immune system to function more reliably [10]. These properties of arabinogalactan were used to create a number of biologically active additives to human food, such as Fibrolar, Probalance, etc. In model experiments, arabinogalactan from larch showed high membranotropy [7]. Because of this, it can be used to increase the absorption of other drugs with low

bioavailability. It was shown that arabinogalactan can serve as a targeted carrier

for the delivery of diagnostic and therapeutic agents, as well as enzymes, nucleic acids, vitamins or hormones, to certain cells, in particular, to hepatocytes (parenchymal cells of the liver). In this case, a complex is formed between the delivered agent and arabinogalactan, which is capable of interacting with the asialoglycoprotein receptor of the cell [8, 9]. The immunological specificity of the polysaccharide macromolecule is directly related to the degree of branching of the galactan core, since immunodeterminant groups are localized on the branched region. Along with the presence of complex branches in the macromolecule, side chains built from L-arabinofuranose residues play an important role. Based on the above literature data and the results of our own research, we have proposed a new modified form of a complex preparation containing natural

herbal ingredients: dihydroquercetin, arabinogalactan and ascorbic acid.

In our study, a reduction in the incidence of influenza and acute respiratory viral infections was observed as a result of taking DHQ-AH-AsC.

Table 1

The incidence of acute respiratory viral infections among people who took and did not take the drug DKV-AG / AsC for prophylactic purposes

ÿlñënö, fóï¿Ü Öíß¿0Ñíñ½ÿê (n)	ÿÜ¡í£íöñ¿á	ÿñluÜÑ dluñ½í long 1 ½ñï.	ÿÜï¿ñ dëÜâó¿í¡öó¡ó	ÿí çñïá dñluÜÑ (2 ½ñï.)
ÿÿÿ/ÿÿ/ÿïÿ n=75	%	2.7	2.7	5.3
	ÿÖÑñ¡ï ~ââñ¡öuzÖÜïöso	1.8	1.4	1.6
		ë > 0.05	ë > 0.05	ë > 0.05
ÿ¿noîñßÜ n=80	%	5.0	3.7	8.7

table 2

Duration of disease and the number of complicated forms among people who took and did not take the drug DKV / AG / AsC

ÿlñnÿnö	ÿïñÇÜ £íßÜ¿ñ¿Ü, ôñ¿.	ÿ£ Öóê ï Üï¿Ü¢ÖñÖó ½ó				ÿëÜÑÜ¿¢óöñ¿áÖÜïöá £íßÜ¿ñcnÖú z ÑÖ ê			ÿßàñ fuï¿Ü ÑÖñú ÖñölÜÑÜïäÜïÜßÖÜïöçço	ÿëñÑÖ Ñ¿óöñ¿áÖÜïöá £íßÜ¿ñçíÖóú
				recital						
				nys.	%					
ÿÿÿ/ÿÿ/ÿïÿ	2	0	0	-	-	...	...		17	8.5
ÿ¿noîñßÜ	four	...	...	2	50.0	...	2	...	37	9.25

After the end of taking the drug, the number of infected people and positive findings in the placebo group was 1.3 times higher (17.8%) than in the group taking the drug (13.6%) (Table 1). These data can be regarded as a positive result of taking the drug on the level of infection. The results of clinical and epidemiological observation showed that taking the drug contributed to a 1.8-fold decrease in morbidity (efficiency index).

Influenza and acute respiratory viral infections lead to a decrease in immunological
body resistance, and, as a result, to WHO
bacterial complications - bronchitis, pneumonia, sinusitis, tonsillitis, etc.

In our observation, during the period of taking the drug, a milder course of diseases was noted, the absence of complicated forms (Table 2). Among those receiving placebo, two cases of complicated course of acute respiratory viral infection were registered - one sinusitis and one case of lacunar tonsillitis, which accounted for 50% of the entire group of patients in the placebo group. This confirms the anti-inflammatory, antiviral and immunomodulatory effects of DHQ-AG-AsC and allows us to recommend it as part of the complex therapy for influenza and other acute respiratory viral infections.

CONCLUSION

The polymeric nature of arabinogalactan, water solubility, permeability through the cell membranes of the body can significantly expand the pharmacokinetic capabilities of drugs immobilized on its matrix - increase bioavailability, change the distribution in the body, prolong the action and increase their selectivity, as well as, in some cases, reduce toxicity. In this case, an increase in the therapeutic effect of the pharmacon can be observed. This opens up a new path for the development of the chemistry of arabinogalactan, associated with the synthesis of physiologically active compounds on its basis. The shown antiviral activity of hydroquercetin also significantly expands the possibilities of using this unique natural antioxidant.

Thus, the creation of complex preparations based on arabinogalactan and dihydroquercetin that combine the unique properties of these natural biologically active substances seems to be very promising.

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