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SUBSTANTIATION OF THE PRESERVATIVE IN THE COMPOSITION OF THE IMMUNOBIOLOGICAL DRUG "CANDIDOCYDE"

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ABSTRACT: Over the last decade the incidence of candidiasis has dramatically increased. To fight candidal infections vaccines with immune modulating properties have been actively investigating in recent years. In our previous studies it was found that the immunobiological drug under the conditional name "Candidocyde" based on the antigens of *C. albicans* and *C. tropicalis* fungi possessed the protective and therapeutic effect. The aim of this work is to substantiate experimentally the preservative in the composition of the immunobiological drug based on the antigens of *C. albicans* and *C. tropicalis* fungi. Various preservatives (phenol, merthiolate and formaldehyde) in the composition of the immunobiological drug have been studied. Compatibility of the preservative and the immunobiological drug was determined by the antibody titers for preventing and treating candidal infections in mice. The results of the ELISA study when preventing candidal infection have shown that after double injection of the immunobiological drug with phenol in the concentration of 0.25 % and merthiolate in the concentration of 0.01 % as preservatives there is 8 times increase the antibody titers. The immunobiological drug with formaldehyde in the concentration of 0.4 % as a preservative provided 8 times increase of the antibody titer. As a result of the research conducted it has been found that formaldehyde in the concentration of 0.4 % reduces the protective effect of the immunobiological drug, while merthiolate in the concentration of 0.01 % and phenol in the concentration of 0.25 % preserves the activity of this drug.

INTRODUCTION: Over the last decade the incidence of candidiasis has dramatically increased, and methods of prevention, diagnosis and treatment currently used cannot provide the proper efficiency[1]. To fight candidal infections vaccines with immune modulating properties have been actively investigating in recent years both in CIS countries and in Europe and America [2].

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It should be noted that currently no domestic vaccine is produced in Ukraine and no imported vaccines for candidiases have been registered. Therefore, development of a vaccine against candidal infection is the topical issue of both domestic and foreign modern pharmacy and medicine.

At the premises of the National University of Pharmacy at the Biotechnology Department and the Department of Microbiology, Virology and Immunology the composition and technology of a potential vaccine have been developed. It is the solution of the immunobiological drug "Candidocyde" based on antigens of *C. albicans*

fungi with the protein concentration of 3 mg/ml and *C. tropicalis* fungi with the protein concentration of 5 mg/ml in the ratio of 1:1 with phosphate buffer solution as a solvent [3]. The antigens of *C. albicans* and *C. tropicalis* fungi were obtained from the biomass of the corresponding fungi using ultrasound [4]. The immunobiological drug developed possesses the immunogenic and therapeutic effect [5, 6].

The immunobiological drug obtained is advisable to produce in reusable bottles for simultaneous immunization of a certain group of people. For multiple liquid medicines, the need for introduction of an effective antimicrobial preservative is determined taking into account the possible contamination during storage and the maximum recommended period of use after opening the container.

Mercurothiolate (merthiolate or thimerosal), formaldehyde and phenol are most often used in vaccines as preservatives [7]. The effectiveness of the preservative may be increased or reduced as a result of interaction with the active substance or other components of the finished product, as well as with packaging or corking material [8].

Data from the scientific literature indicate that many of the preservatives are corrosive substances in relation to biological objects; therefore, before using them it is necessary to check the effect of preservatives on the activity of the immunobiological drug.

The aim of this work is to substantiate experimentally the preservative in the composition of the immunobiological drug based on the antigens of *C. albicans* and *C. tropicalis* fungi.

MATERIAL AND METHODS:

The immunobiological drug based on the antigens of fungi of *C. albicans* with the protein concentration of 3 mg/ml and *C. tropicalis* with the protein concentration of 5 mg/ml in the ratio of 1:1 was studied with various preservatives: 0.25 % phenol, 0.01 % merthiolate and 0.4 % formaldehyde. When choosing a preservative the requirements for this group of substances were taken into account, namely activity against a wide spectrum of microorganisms, slow formation of resistant variants of microorganisms, demonstration of antimicrobial properties in a wide pH range, compatibility with the main components of the dosage form, safety. To reveal the effect of a preservative on the activity of the immunobiological drug the experimental samples consisting of the immunobiological drug solution and preservatives studied in the maximum allowable concentration were obtained.

To determine the drug immunogenicity when preventing and treating candidiases the experiments were carried out in healthy two month white mice weighing 18 - 22 g. There were six animals in the control and experimental groups each; they were kept in the same conditions on a standard diet. All studies on animals were conducted with permission of the Ethical Committee. Before the research the animals acclimatized themselves under experimental room conditions. In studies regarding prevention of candidal infection the mice received a double intramuscular injection of 0.2 ml of the solution “Candidocyde” in the upper part of the rear right paw with an interval of 14 days. After immunization the animals were infected intraperitoneally. For this purpose the suspensions of *Candida albicans* fungi in the amount of 20 mln. of cells and *C. tropicalis* in the amount of 60 mln. of cells in the volume of 1 ml were introduced with an interval of 1 h.

The sterile 0.9 % isotonic saline solution was injected to the animals of the control group. In 14 days the protective functions of the organism of animals were determined by the titer of specific antibodies of *C. albicans* when conducting enzyme immunoassay (ELISA) according to the SPhU, I-st ed., art. 2.7.1, p.55-57. For this purpose the reagent kit for immunoassay detection of antibodies of class G to *C. albicans* using the test system ELISA "Vector-best" made in Russia was applied. In the absence of sets of reagents for immunoassay detection of antibodies of class G to *C. tropicalis*, the identification of antibodies only of class G to *C. albicans* was conducted.

When conducting the research on therapy the animals were infected intraperitoneally with the suspensions of *Candida albicans* in the amount of

20 mln. of cells and *Candida tropicalis* in the amount of 60 mln. of cells in the volume of 1 ml introduced with the interval of 1 hour. In 5 days a double intramuscular injection of 0.2 ml of the solution "Candidocyde" was introduced to mice in the upper part of the rear right paw with an interval of 14 days. The animals of the control group were injected the sterile 0.9 % isotonic saline solution. In 14 days the protective functions of the organism of animals were determined by the titer of specific antibodies of *C. albicans* using ELISA.

To obtain the reliable results of the study all data were statistically processed, the median for all groups and its confidence interval were calculated. Statistical processing of the digital data obtained was performed using the package of the Statistika applied software package.

RESULTS AND DISCUSSION: The results of the ELISA study when preventing candidal infection have shown that after double injection of the immunobiological drug with phenol in the concentration of 0.25 % and merthiolate in the concentration of 0.01 % as preservatives there is 8 times increase in the antibody titers compared to the antibody titers in animals prior to the research. The antibody titers obtained were stored for 3 months. The immunobiological drug with formaldehyde in the concentration of 0.4 % as a preservative provided 8 times increase of the antibody titer in 1 month, and double increase in 3 months. The results of the research are given in **Table 1.**

TABLE 1: THE ANTIBODY TITERS WHEN INTRODUCING THE IMMUNOBIOLOGICAL DRUG WITH THE PRESERVATIVES STUDIED IN PREVENTION OF CANDIDIASES

Preservatives	Concentration, %	The antibody titers in ELISA for *C. albicans*		
		Prior the research	Results in 1 month	Results in 3 months
Phenol	0.25	1:350 ± 102	1:2800 ± 752	1:2800 ± 761
Merthiolate	0.01	1:400 ± 97	1:3200 ± 763	1:3200 ± 775
Formaldehyde	0.4	1:300 ± 93	1:2400 ± 754	1:600 ± 343
Control	-	1:350 ± 95	1:2400 ± 754	1:2400 ± 741

Note. Control – the immunobiological drug without the preservative, n=6, P<0.5

The results of the ELISA study when treating candidal infection have shown that after double injection of the immunobiological drug with all preservatives there is 8 times increase in the antibody titers compared to the antibody titers in animals prior to the research. The results of the research are given in **Table 2**.

TABLE 2: THE ANTIBODY TITERS WHEN INTRODUCING THE IMMUNOBIOLOGICAL DRUG WITH THE PRESERVATIVES STUDIED IN TREATMENT OF CANDIDIASES

Preservatives	Concentration, %	The antibody titers in ELISA for *C. albicans*		
		Before infection	After infection	After treatment
Phenol	0.25	1:350 ± 97	1:700 ± 361	1:2800 ± 754
Merthiolate	0.01	1:400 ± 102	1:800 ± 356	1:3200 ± 768
Formaldehyde	0.4	1:400 ± 94	1:800 ± 362	1:3200 ± 764
Control	-	1:400 ± 93	1:700 ± 357	1:3200 ± 763

Note. Control – the immunobiological drug without the preservative, n=6, P<0.5

It should be noted that phenol is a carcinogenic substance, however, its amount in a single dose with the volume of 0.2 ml is extremely low. Merthiolate is an organic salt of mercury, but it does not contain pure mercury. In addition, phenol and merthiolate have shown good results as preservatives for vaccines over a number of years. Therefore, phenol and merthiolate were selected for further research.

CONCLUSIONS:

The results of studying the samples activity of the immunobiological drug based on the antigens of fungi of *C. albicans* with the protein concentration of 3 mg/ml and *C. tropicalis* with the protein concentration of 5 mg/ml in the ratio of 1:1 indicate that formaldehyde in the concentration of 0.4 % reduces the protective effect of the immunobiological drug, while merthiolate in the concentration of 0.01 % and phenol in the

concentration of 0.25 % preserve the activity of this drug.

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