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## Research of low molecular fraction of *C. albicans* fungus cells by the ELISA in subcutaneous administration

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### ABSTRACT

The purpose of this work is a study of low molecular weight fraction with *C. albicans* protein concentrations of 1, 2, 3, and 4 mg/ml by antibody titers in subcutaneous administration. A low molecular fraction of *C. albicans* proteins in concentration 1, 2, 3, 4, and 5 mg/ml has been studied in white mice. Mice have subcutaneously injected 0.2 ml of the test fraction into the upper part of the right hind paw. After 14 days, 0.2 ml of test fractions were injected repeatedly into the upper part of the left hind paw. 1 month for one group and 3 months for the second group of experimental animals after the second injection intraperitoneal infection of the animals was carried out. After 14 days, determination of the protective functions of the animal body by titer of *C. albicans* specific antibodies has been performed by enzyme-linked immunoassay. Studies have shown that the low molecular weight fraction of *C. albicans* fungi cells antigens at the double subcutaneous injection of 0.2 ml does not provide the activation of immune mechanisms. The low molecular weight fraction of *C. albicans* proteins at subcutaneous administration does not activate the body's defense mechanisms in preventing candidiasis.



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### INTRODUCTION

Among mycotic infections, candidiasis occupies one of the leading places. (Argenio and Wilson, 2010) The incidence of candidiasis is increasing worldwide, and it is associated with the widespread use of antibacterials, hormones, cytostatics, other drugs and an increase in the incidence spectrum, which creates a positive background for the development of candidiasis (diseases of the hematopoietic organs,

immunodeficiency states, malignant tumors, radiation damage, HIV infection, etc.) (Pfaller and Diekema, 2007; Wack and Rappuoli, 2005). Candidiasis agents belong to yeast-like fungi of the genus *Candida*, family — Cryptococcaceae and include, according to various sources, 134 species, of which approximately 27 are seeding from the affected areas of patients (Cutler et al., 2007). The etiologic agents of the disease most often are (in descending order): *C. albicans*, *C. tropicalis*, *C. krusei*, and others (Bromuro et al., 2002).

The absence of rapid, sensitive, and specific methods of invasive mycosis diagnosis is a serious drawback in the treatment of such patients, and many of these individuals die before receiving adequate therapy (Daan et al., 2008; Roemer and Krysan, 2014). Therefore, in our time, resort to blind antifungal therapy, that is without establishing an accurate diagnosis of fungal infection. Rational, empirical antifungal therapy is the treatment of invasive fungal infection at the earliest stage of a patient's disease when there is a high risk of complications

of such an infection (Segal *et al.*, 2006). However, the choice of antifungal agents between fluconazole, some azoles, and polyene amphotericin B is small. Besides, there is a lot of data regarding the loss of sensitivity of *Candida* genus fungi to traditional antifungal drugs that have been in use for decades (Rybalkin *et al.*, 2020).

Vaccines to combat candidiasis with immunomodulatory properties have been actively investigated in recent years, both in the CIS countries and in Europe and America. (Nabel, 2013; Guinea, 2014). Vaccines are developed using biotechnological and immunological approaches (Miceli *et al.*, 2011; Pfaller and Diekema, 2004). It should be noted that at the moment in Ukraine, there is no domestic vaccine produced and no imported vaccine is registered for the prevention and treatment of candidiasis. On this basis, the development of a vaccine against candidiasis infection is an urgent issue in modern medicine and pharmacy. Considering that most often the causative agents of candidiasis are different types of fungi of the genus *Candida*, it is appropriate to develop an associated vaccine based on the most common species, namely *C. albicans* and *C. tropicalis*.

Based at the Biotechnology and Microbiology, Virology and Immunology Department of the National University of Pharmacy, the authors have developed a method for the disintegration of *Candida* fungal cells using ultrasonic radiation. The composition of the extract-disintegrate of *Candida* cells includes proteins and polysaccharides that possess antigenic properties. In this case, according to the requirements of the SPU, the identification of the active substance is carried out in terms of protein. Previously, studies were conducted to determine the effectiveness of subcutaneous and intramuscular injections of *C. albicans* fungus cells disintegrate solution with a molecular mass of antigens greater than 10 kDa in an animal experiment for prevention and treatment of candidiasis infection.

The studies have found that the antigens of this fraction show immunogenic properties at *C. albicans* fungus proteins concentration of 3 mg/ml at intramuscular administration and do not exhibit immunogenic properties at subcutaneous administration. Now it is advisable to conduct a study of low molecular weight fractions less than 10 kDa with *C. albicans* protein concentrations of 1, 2, 3, and 4 mg/ml for immunogenicity at subcutaneous and intramuscular injection by antibody titers in the prevention and treatment of candidiasis. The purpose of this work is a study of low molecular weight fraction less than 10 kDa with *C. albicans* protein

concentrations of 1, 2, 3, and 4 mg/ml by antibody titers in the prevention of candidiasis in subcutaneous administration.

## MATERIALS AND METHODS

*C. albicans* fungal cells were cultured in test tubes on Sabouraud agar at  $25 \pm 2$  °C for 48 hours and washed the fungal cells with sterile isotonic 0.9% sodium chloride solution. The resulting suspensions of *C. albicans* fungal cells were transferred to Sabouraud agar mattresses incubated at  $25 \pm 2$  °C for 6 days and washed the fungal cells with sterile isotonic 0.9% sodium chloride solution. Determined microbiological purity by microscopy of the suspension of *C. albicans* fungal cells and standardized them for the specific content of fungal cells per unit volume of isotonic 0.9% sodium chloride solution, by counting fungal cells in the Goryaev chamber.

From the obtained *C. albicans* cells, isolated proteins and polysaccharides using an ultrasonic disintegrator at a wavelength of 22 kHz and an exposure of 15 min. Filtered through the membrane "Vladipore" MFA - MA No. 3, which provides filtration of biological material with a size of 10 kDa. Two fractions were obtained: the first — with a molecular size of less than 10 kDa and the second one the size of the molecules more than 10 kDa. The study used a low molecular weight fraction of less than 10 kDa. Next, pre-filtration was performed using filters with a pore diameter of 0.45  $\mu$ m and sterilizing filtration using filters with a pore diameter of 0.22  $\mu$ m.

The resulting fractions were evaluated for effectiveness in the prevention of candidiasis in experiments on healthy white mice of two months of age weighing 18–22g of 10 animals in the control and experimental groups, which were kept under standard diet in the same conditions. Before the study, the animals were acclimated to the experimental room. The mice were injected subcutaneously in the upper part of the right hind paw with 0.2 ml of the studied fraction with a concentration of *C. albicans* protein 1, 2, 3, and 4 mg/ml. These concentrations were determined in previous studies for a fraction with molecular size greater than 10 kDa.

After 14 days, 0.2 ml of test fractions were injected repeatedly into the upper part of the left hind paw. Animals in the control group were administered saline. 1 month for one group and 3 months for the second group of experimental animals after the second injection intraperitoneal infection of the animals was carried out. For this purpose, a suspension of *C. albicans* fungi was used in the amount of 20 million cells in a volume of 1 ml. After 14 days, deter-

**Table 1: Immunogenicity study of low molecular weight fraction of *C. albicans* fungal antigens in the prevention of candidiasis at the subcutaneous injection**

The route of administration	Antigens of <i>C. albicans</i> , protein concentration, mg / ml				
	1	2	3	4	Control
	<b>Antibody titers</b>				
	<b>After the 1st injection</b>				
s.c.	1:(800±34)	1:(400±17)	1:(600±28)	1:(800±34)	1:(300±14)
	<b>After the 2nd injection</b>				
s.c.	1:(1000±45)	1:(600±27)	1:(1600±71)	1:(1600±72)	1:(200±10)
	<b>After 1 month</b>				
s.c.	1:(1000±43)	1:(800±33)	1:(1200±55)	1:(1600±68)	1:(400±17)
	<b>After 3 months</b>				
s.c.	1:(800±35)	1:(800±34)	1:(1600±70)	1:(600±71)	1:(300±13)

n =10, P&lt;0.05

ination of the protective functions of the animal body by titer of *C. albicans* specific antibodies has been performed by enzyme-linked immunoassay as described in the state pharmacopoeia of Ukraine Ed. 1, §2.7.1, p.55-57. For this purpose, a set of reagents for enzyme-linked immunosorbent detection of class G antibodies to *C. albicans* has been utilized using the Vector-Best ELISA test system.

## RESULTS AND DISCUSSION

Before the study, in the animals of the control and experimental groups antibody titers to *C. albicans* fungi were determined, which were in the range 1: 200-1: 400. This can be explained by the contact of the animals with the pathogen in the process of life or carriage of the fungus since it is a part of the normal microflora of animals. The results of previous studies by enzyme-linked immunosorbent assay in the prevention of candidiasis infection have shown that after two subcutaneous injections of the high molecular weight fraction of antigens of *C. albicans* with protein concentrations of 1, 2, 3, and 4 mg/ml with an interval of 14 days there was no increase in antibody titers, which were within 1: 800-1: 1600 after 1 and 3 months. When using a low molecular weight fraction of *C. albicans* fungi antigens with a protein concentration of 1, 2, 3, and 4 mg/ml, which was administered by the same scheme as the high molecular weight fraction of *C. albicans* fungi antigens, antibody titers were also in the range of 1: 800-1: 1600 after 1 and 3 months. The antibody titers in the control group were in the range of 1: 200-1: 400. The results are shown in Table 1.

The results of the studies indicate that antigens of the low molecular weight fraction of *C. albicans* fungus do not activate the protective mechanisms dur-

ing subcutaneous administration, as well as antigens of the high molecular weight fraction of *C. albicans* fungus with subcutaneous administration from previous studies. Comparing the results obtained in previous and present studies, it is safe to say that the intramuscular administration of the high molecular weight fraction of *C. albicans* fungus antigens more strongly stimulates the formation of antibodies responsible for humoral immunity. Based on the data obtained, it can be argued that the high molecular weight fraction of *C. albicans* fungus antigens at intramuscular injection can be a potential antigen for the development of vaccines for the prevention and treatment of candidiasis, in contrast to the low molecular weight fraction at subcutaneous injection.

## CONCLUSIONS

Studies have shown that the low molecular weight fraction of *C. albicans* fungi cells antigens with protein concentrations of 1, 2, 3 and 4 mg/ml at the double subcutaneous injection of 0.2 ml does not provide the activation of immune mechanisms in candidiasis prevention. For further studies, it is advisable to use a high molecular weight fraction of *C. albicans* fungus cells antigens with a protein concentration of 3 mg/ml at intramuscular injection, based on which it is planned to develop a vaccine to prevent and treat candidiasis.

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The authors declare that they have no funding support for this study.

## Conflict of Interest

The authors declare that they have no conflict of interest for this study.

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