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# Research of antibody titres to antigens of a low molecular fraction of C. Albicans fungus disintegrate at preventing candidomicosis

Mykola V Rybalkin[1], Tatiana V Diadiun[*2], Olga S Kalyuzhnaya[1], Svitlana M Kovalenko[2], Olexandr S Kukhtenko[3]

[1]Department of Biotechnology, National University of Pharmacy, 61002, 53, Pushkinska str., Kharkov, Ukraine
[2]Department of Commodity science, National University of Pharmacy, 61002, 53, Pushkinska str., Kharkov, Ukraine
[3]Technologies of pharmaceutical preparations department, National University of Pharmacy, 61002, 53, Pushkinska str., Kharkov, Ukraine

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ABSTRACT

The purpose of this work is the study of antibody titers of low molecular weight proteins fractions less than ten kDa with C. Albicans protein concentrations of 1, 2, 3 and 4 mg/ml in the prevention of candidiasis. A low molecular fraction of C. Albicans proteins in concentration 1, 2, 3, 4 and 5 mg/ml were examined in white mice. The mice were intramuscularly injected into the upper part of the right hind paw 0.2 ml of the test fractions with the investigated protein concentrations. After 14 days, 0.2 ml of test fractions were injected again into the upper part of the left hind paw. Animals in the control group were administered saline. After one month for one group and after three 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, determination of the protective functions of the animal body by the titer of specific C. Albicans antibodies has been performed during enzyme-linked immunoassay. 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 a double intramuscular injection of 0.2 ml does not provide activation of immune mechanisms. The low molecular weight fraction of C. Albicans proteins does not activate the body's defence mechanisms.

[*]Corresponding Author

Name: Tatiana V Diadiun
Phone: +380996505417
Email: diadiunscience@gmail.com

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## INTRODUCTION

Invasive fungal diseases affect humans when natural defence mechanisms weaken. These infections often develop in a hospital setting. For example, candidemia, an infection caused by one of several Candida fungi. It usually develops after the healthy bacterial microflora of the patient is "wiped out" by antibiotics on the surface of the skin and mucous membranes, disturbed by central venous catheters or surgery (Carvalho *et al.*, 2012; Diekema *et al.*, 2012). The candidemia ranks fourth among the most common bloodstream infections in hospitalized patients, both in the United States and in

many European countries. And the mortality from such Candida infections remains around 30-40%, even after antifungal therapy (Hani *et al.*, 2015; Cassone and Casadevall, 2012; Han and Rhew, 2012). Given the increasing incidence and unacceptably high rates of morbidity and mortality, the prevention of invasive fungal infections has become a matter of paramount importance.

In recent years, several research groups around the world have focused on the creation of a vaccine against candidiasis (Zaharieva *et al.*, 2019; Edwards, 2012; Rybalkin *et al.*, 2014), one of the most severe and deadly fungal infections.

It should be noted that at the moment in Ukraine, there are no domestic or imported vaccines for the prevention and treatment of candidiasis (Daele *et al.*, 2019). On this basis, the development of a vaccine against candidiasis infection is an urgent issue in modern medicine and pharmacy.

The authors have developed a method for the disintegration of Candida fungal cells using ultrasonic radiation at the Biotechnology, Microbiology, and Immunology Department of the National University of Pharmacy. The composition of the extract-disintegrate of Candida cells includes proteins and polysaccharides that have antigenic properties. According to the requirements of the SPU, determination of the active substance is carried out on the protein.

Previously, studies were conducted to determine the effectiveness of the solution of C. Albicans cells disintegrate with a molecular weight of antigens higher than ten kDa in an animal experiment for the prevention and treatment of candidiasis. Research has found that the antigens of this fraction show immunogenic properties at C. Albicans protein concentration of 3 mg/ml. Now it is advisable to conduct a study of low molecular weight fractions less than ten kDa with C. Albicans protein concentration of 3 mg/ml for immunogenicity by antibody titers in the prevention and treatment of candidiasis.

The purpose of this work is the study of antibody titers of low molecular weight proteins fractions less than ten kDa with C. Albicans protein concentrations of 1, 2, 3 and 4 mg/ml in the prevention of candidiasis.

## MATERIALS AND METHODS

Fungal cells were cultured in test tubes on Saburaud 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 Saburaud agar mattresses incubated at 25 $\pm$ 2° C for six days and washed the fungal cells with sterile isotonic 0.9% sodium chloride solution. Purity has been determined 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 fungal cells isolated proteins using an ultrasonic disintegrator at a wavelength of 22 kHz and an exposure of 15 min (Abashina *et al.*, 2018). It was filtered through the membrane "Vladipore" MFA - MA No. 3, which provides blocking of biological material with a size of 10 kDa. Two fractions were obtained: the first - with a molecular size of less than ten kDa and the second one the size of the molecules more than ten kDa. The study used a low molecular weight fraction of less than ten 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 received fractions were evaluated for effectiveness in the prevention of candidiasis in experiments on healthy white mice of two months of age weighing 18 - 22 years 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 acclimatized in the trial room. Mice were intramuscularly injected into the upper part of the right hind paw with 0.2 ml of test fractions with C Albicans protein concentration 1, 2, 3 and 4 mg/ml. These concentrations were determined in previous studies for a portion with a molecular size more than ten kDa. After 14 days, 0.2 ml of test fractions were injected again into the upper part of the left hind paw. Animals in the control group were administered saline.

After one month for one group and after three 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, determination of the protective functions of the animal body by the titer of specific C. Albicans antibodies was performed during enzyme-linked immunoassay according to SPU Ed. 1, §2.7.1, p.55-57. For this purpose, a set of reagents for the enzyme-linked immunosorbent detection of G antibodies to C. Albicans was used using the Vector-Best ELISA test system.

 

**Table 1: Study of immunogenicity of low molecular weight fraction of C. albicans fungal antigens in the prevention of candidiasis**

| The path of introduction | C. albicans fungus antigens, protein concentration, mg / ml | | | | |
|---|---|---|---|---|---|
| | Antibody titers | | | | |
| | 1 | 2 | 3 | 4 | Control |
| | After the 1st injection | | | | |
| I.m. | 1: (800 ± 35) | 1: (800 ± 34) | 1: (800 ± 33) | 1: (800 ± 34) | 1: (400 ± 89) |
| | After the 2nd injection | | | | |
| I.m. | 1: (1600 ± 65) | 1: (1600 ± 71) | 1: (1600 ± 68) | 1: (800 ± 35) | 1: (200 ± 42) |
| | After 1 month | | | | |
| I.m. | 1: (1600 ± 72) | 1: (1600 ± 67) | 1: (1600 ± 71) | 1: (800 ± 34) | 1: (400 ± 95) |
| | After 3 months | | | | |
| I.m. | 1: (1600 ± 65) | 1: (1600 ± 72) | 1: (1600 ± 68) | 1: (800 ± 36) | 1: (400 ± 92) |

Note: n = 10, P <0.05

## RESULTS AND DISCUSSION

In animals of the control and experimental groups antibody titers to fungi C. Albicans have been determined, which were in the range 1: 200-1: 400. It can be explained by the contact of animals with the pathogen in the process of life or carriage of the fungus since it is a part of the healthy microflora of animals.

The results of previous enzyme-linked immunosorbent assay studies in the prevention of infection have shown that after two injections of the high molecular weight fraction of antigens of C. Albicans fungi with a protein concentration of 3 mg/ml with an interval of 14 days there is an increase in antibody titers, which was in the range 1: 1600-1: 4000 after 1 and 3 months.

When using a low molecular weight fraction of antigens of fungi C. Albicans with a protein concentration of 1, 2, 3 and 4 mg/ml, administered according to the same scheme as high molecular weight fractions of antigens of fungi C. Albicans, antibody titers were in the range 1: 800-1: 1600 after 1 and 3 months. The antibody titers in the control group were in the range 1: 200-1: 400 (Table 1).

Thus comparing the results obtained in the experimental and control groups, it is safe to say that the introduction of a 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 may be potential antigens for vaccine development in the prevention and treatment of candidiasis.

## CONCLUSION

Studies conducted 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 a double intramuscular injection of 0.2 ml does not provide activation of immune mechanisms. For further studies, it is advisable to use high molecular weight fraction of C. Albicans fungi cells antigens with a protein concentration of 3 mg/ml, based on which it is planned to develop a vaccine for the prevention and treatment of candidiasis infection.

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**Conflict of Interest**

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

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