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Research Article

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Determination of toxicity of the immunobiological drug for prevention and treatment of candidal infection

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ABSTRACT

In recent years the increased incidence of candidal infection is observed in the world, among them candidiasis takes the leading place. Candidiasis is an infectious disease caused by yeast-like fungi of Candida genus. There are both mild forms of this disease – candidiasis of the skin and mucous membranes and very severe forms – generalized candidiasis of visceral organs. In order to fight candidiasis the authors have developed an immunobiological drug for prevention and treatment of candidal infection. The drug contains antigens of C. albicans and C. tropicalis fungi with the total protein concentration of 4 mg/ml. The composition of the drug also includes the solvent phosphate buffer solution with pH 7.2 ± 0.2 and the preservative phenol in the concentration of 0.25 %. The drug developed possesses the protective and therapeutic effect. The aim of this work was to study toxicity of the immunobiological drug. The abnormal toxicity of the solution of the immunobiological drug for prevention and treatment of candidal infection was studied in two species of animals – mice and guinea pigs. To assess the toxic effect of the immunobiological drug solution on the animals' organism the study of dynamics of the animals' body weight was performed. The results obtained were compared to the body weight dynamics of the intact group that received the phosphate buffer solution. The animals were observed within 14 days. After the end of the observation period the autopsy and macroscopic examination of the animals' internal organs were performed. During the autopsy when examining the internal organs of the animals the signs of intoxication or other manifestations of pathological processes were not revealed. To identify pathology the mass coefficients of the animals' internal organs were determined. As a result of the research conducted it has been found that the immunobiological drug developed for prevention and treatment of candidal infection is atoxic.

Key words: candidiasis, antigen, vaccine, immunity, protein

INTRODUCTION

In recent years the increased incidence of candidal infection is observed in the world, among them candidiasis takes the leading place. Candidiasis is an infectious disease caused by yeast-like fungi of *Candida* genus. There are both mild forms of this disease – candidiasis of the skin and mucous membranes and very severe forms – generalized candidiasis of visceral organs [1-2].

C. albicans has a high sensitivity to antifungal drugs, at the same time resistance to them can be rapidly developed. In recent years due to the active use of fluconazole the fungi of *Candida* genus are often insensitive to it. Another world problem is increasing prevalence of different species of *Candida* genus fungi (non-albicans), the part of which from the very beginning is insensitive to azoles, but it is common practice to start the treatment with them [3-4].

To fight candidal infection in recent years vaccines with the immunomodulatory properties are being actively investigated both in the CIS countries and in the countries of Europe and America [5-7]. It should be noted that

currently no domestic vaccine is produced in Ukraine and no imported vaccines have been registered for prevention and treatment of candidiases. Therefore, development of a vaccine against candidal infection is the topical issue of modern medicine and pharmacy.

In order to fight candidiasis the authors have developed an immunobiological drug for prevention and treatment of candidal infection. The drug contains antigens of *C. albicans* and *C. tropicalis* fungi with the total protein concentration of 4 mg/ml. The composition of the drug also includes the solvent phosphate buffer solution with pH 7.2 ± 0.2 and the preservative phenol in the concentration of 0.25 %. The drug developed possesses the protective and therapeutic effect [8].

The aim of our work is to study toxicity of the immunobiological drug for prevention and treatment of candidal infection.

EXPERIMENTAL SECTION

The test on the abnormal toxicity was performed according to the method of the SPhU 2.6.9. The abnormal toxicity of the solution of the immunobiological drug for prevention and treatment of candidal infection was studied in two species of animals – mice and guinea pigs. Before the research the animals acclimatized themselves under experimental room conditions. 0.2 Ml of the immunobiological drug with the protein concentration of 4 mg/ml was introduced intraabdominally to five white mice weighing 18 - 22 g and two guinea pigs weighing 200 - 300 g and being on a standard diet. The animals were observed within 14 days.

To assess the toxic effect of the immunobiological drug solution on the animals' organism the study of dynamics of the animals' body weight was performed. The results obtained were compared to the body weight dynamics of the intact group that received the phosphate buffer solution. The animals were observed within 14 days.

After the end of the observation period the autopsy and macroscopic examination of the animals' internal organs were performed. During the autopsy when examining the internal organs of the animals the signs of intoxication or other manifestations of pathological processes were not revealed. To identify pathology the mass coefficients of the animals' internal organs were determined.

RESULTS AND DISCUSSION

The results of the test on abnormal toxicity are given in Table 1.

Table 1 The study of the abnormal toxicity of the solution of the immunobiological drug

Protein concentration, mg/ml	Death of animals / the number of animals	
	Mice	Guinea pigs
4	0/5	0/2

The animals were tidy, active and with a good appetite, they reacted to sound and light stimuli, the processes of urination and defecation were within the norm, respiratory disorders and convulsions were not observed. The reflex excitability of animals was within the norm. The animals' skin was also within the norm. All animals were active by the end of the experiment. The animals' death was not observed.

The results of the assessment of the toxic effect of the immunobiological drug solution on the animals' organism as the study of dynamics of the animals' body weight are given in Table 2.

Table 2 Dynamics of the animals' body weight

Species and gender of animals	Groups of animals	Dynamics of the body weight, g		
		Initial data	3 days	7 days
Mice (females)	Test group	22.41±0.43	22.67±0.47	24.43±1.15
	Intact control	21.37±0.62	22.41±0.54	23.89±0.78
Guinea pigs (females)	Test group	238.72±7.65	239.35±8.27	241.42±9.35
	Intact control	237.51±7.62	240.47±8.54	242.34±9.62

Note. Mice n=5, guinea pigs n=2, P<0.5.

The study of the body weight in dynamics has demonstrated that within the period of observation the insignificant positive variations in the weight take place in the groups of animals after intraperitoneal introduction of the immunobiological drug solution. However, these indices do not have statistically significant differences from those

of the group of intact control.

After the end of the observation period (14 days) the autopsy and macroscopic examination of the animals' internal organs were performed. During the autopsy when examining the internal organs of the animals the signs of intoxication or other manifestations of pathological processes were not revealed. By their size, colour, consistency, and location the animals' internal organs were within the norm and did not differ from the internal organs of the intact animals group. As for the mass coefficients of the animals' internal organs the apparent pathology has not been found. The data of the mass coefficients of the animals' internal organs have proven it. The research results are given in Table 3.

Table 3 The mass coefficients of the animals' internal organs when studying the abnormal toxicity of the immunobiological drug solution

Parameter		The mass coefficient of the organ, %	
		Vaccine	Control
		Mice	
Liver		4.71±0.14	4.45±0.17
Kidneys	right	0.53±0.03	0,52±0,03
	left	0.52±0.03	0,51±0,04
Lungs		0.85±0.07	0.86±0.05
Adrenal glands		0.07±0.03	0.06±0.02
Heart		0.45±0.02	0.47±0.02
Spleen		0.68±0.07	0.86±0.21
Thymus		0.30±0.03	0.29±0.03

Note. n=6, P<0.5.

According to the literature, polysaccharides of *C. albicans* are really non-toxic, while glycoproteins possess somewhat greater toxicity. Probably, our research results can be explained by the detoxifying effect of ultrasound known from the literature. As early as in 1955 Tarnasi demonstrated that the action of ultrasound for 2-4 h (835 khz/s) partially detoxified O- and Уi-antigens of typhoid bacillus without notable changes of their serological and immunological properties. Further the same action of ultrasound has been confirmed by many investigations concerning various types of microorganisms. A. Barone with co-authors have not found toxic substances for mice in the extracts of *C. albicans* obtained by ultrasound.

Analysis of the results was performed by the methods specified in the SPhU, monograph 5.3. «*Statistical analysis of the results of biological tests and assays*». The research on the study of the abnormal toxicity has proven that the immunobiological drug developed does not exhibit toxicity.

CONCLUSION

As a result of the research conducted it has been found that the immunobiological drug developed for prevention and treatment of candidal infection based on antigens of *C. albicans* and *C. tropicalis* fungi with the total protein concentration of 4 mg/ml together with the solvent phosphate buffer solution at pH 7.2 ± 0.2 and the preservative phenol in the concentration of 0.25 % is atoxic.

REFERENCES

[1] ОА Капустина; ЛЕ Логачева; ОЛ Карташова, *Известия Оренбургского государственного аграрного университета*, **2009**, 4 (24), 179–181.
[2] НА Апанасенко, *Иммунопатология, аллергология, инфектология*, **2010**, 1, 146-147.
[3] SL Gut-Landman; M Wutrich; T Hohl, *Curr. Opin. Immunol*, **2012**, 24, 1–10.
[4] PG Pappas, *Infect. Dis. Clin. North Am.*, **2006**, 20, 485–506.
[5] НИ Глушко; РЛ Смирнова; ЕВ Агафонова; ВП Нефедов, Соврем, микология в России, Москва издательство, **2002**, 354-355.
[6] JE Domer; RE Garner; RN Befidi-Mengue, *Infect. Immun*, **1989**, 57, 693-700.
[7] PG Pappas, *Proc. Natl Acad. Sci. USA*, **2008**, 105, 13526–13531.
[8] MV Rybalkin, NI Filimonova, OP Strilets, LS Strelnikov *Journal of Chemical and Pharmaceutical Research*, **2014**, 6, 4, 954-957.