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THE EFFECT OF CHLOROPHYLLIPTUM SOLUTION ON THE ADHESIVENESS OF CANDIDA SPP

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The purpose of this study was to investigate the adhesive properties of clinical strains of *C.albicans, C.glabrata* and the effect of Chlorophylliptum solution on their adhesion process. The study was carries out on 5 clinical isolates of *C. albicans* and 5 isolates of *C.glabrata* taken from patients of the Surgery unit. Adhesive potential of the studied clinical strains of microorganisms were evaluated by using the standard method of Brillis V.I. et al. We used an alcoholic solution of Chlorophylliptum thick extract as an active agent and 96% ethanol as an additive ("Halychpharm" corporation, Lviv, Ukraine, No. UA / 4551/02/01 dated October, 31, 2016). The subfungistatic concentrations used in the course of our research was ¼ of fungistatic concentration of the studied extract for each type of pathogen. It was found out that the subfungistatic concentrations of alcohol solution of Chlorophylliptum extract did not affect the adhesive properties of clinical strains of *C.albicans.* In turn, the Chlorophylliptum extract demonstrated considerable inhibitory effect on the ability of clinical isolates of *C. glabrata* to adhere to human erythrocytes.

Key words: *C.albicans, C. glabrata,* adhesiveness, Chlorophylliptum.

The work is a fragment of the research project "Study of the role of opportunistic and pathogenic infectious agents with different sensitivity to antimicrobial and antiviral drugs in human pathology", state registration No. 0118U004456.

Opportunistic fungi of the genus Candida are known as a constituent of the normal microflora of mucous membranes in the human body. At physiological homeostasis, these opportunistic fungi do not initiate progression of infectious processes. However, any immunodeficient condition can lead to the development of candidiasis. Therefore, nowadays medical researchers and clinicians are facing the constant growing of infectious diseases involving fungi of the genus Candida. Recently, there have been a number of reports pointing out the increase in the prevalence of systemic candidiasis. This can be due to lowered population immunity, the development of new medical invasive technologies and procedures, expansion of antibiotic-resistant types of fungi [9].

In the overwhelming majority of cases of candidiases, *C. albicans* has been confirmed as an important pathogen [6]. The main factor determining virulence of this species is its powerful invasive activity due to the formation of hyphal tubes, biofilms, and the presence of specific cell wall proteins [12].

For the past decade, the global medical community has reported the growth of non-albicans infections caused by the genus Candida, e.g.: *C.tropicalis, C.parapsilosis, C.glabrata* and *C.krusei*. Among the species mentioned, *C.glabrata* plays the leading role in the occurrence rate of non-albicans disease. This pathogen has the same generic name, but phylogenetically is different from *C.albicans*. Findings of sequence analyses have demonstrated that its genome is closer to *S. cerevisiae* and this gives it biological properties different from other members of the genus *Candida* [6, 7].

In recent years, there have been a large number of studies investigating the virulence factors of *C.glabrata* and their impact on the pathogenesis of diseases caused by *C.glabrata*. Vale-Silva and Sanglard affirm that adhesive capacity is the major virulence factor.

The difference in the evolutionary development of these two types of microorganisms was reflected not only in the pathogenesis, but also in sensitivity to antimycotic agents. For instance, *C. albicans* is sensitive to azole antifungal compounds, while *C.glabrata* is found to be resistant [8]. During the therapy with azole compounds, a latent fungal infectious disease develops and at the same time the *C.glabrata* populations accumulate and penetrate into deep tissue producing no marked clinical manifestations [6]. This rouses researchers to search for alternative means of the therapy, which will not only possess antimycotic properties, but also will have an effect on individual links of pathogenesis [3].

We focus our attention on the alcoholic extract of Eucalyptus globulus produced in Ukraine under the trade name "Chlorophylliptum". The disposal drug instructions states the medicine may be used for the treatment and prevention of infections caused by *Staphylococcus aureus* [11]. Currently, this herbal extract is widely used in clinical practice as an antibacterial agent [1, 2, 13, 15].

Considering the significant development of resistance to antifungal drugs among *C. glabrata* and the prevalence of adhesiveness as their main factor of pathogenicity, the development of alternative drugs having fungicidal properties and inhibiting the adhesive ability of microorganisms and their implementation into clinical practice is among the most relevant and promising issues for research [3].

The purpose of the study was to investigate the adhesive properties of clinical strains of *C.albicans, C.glabrata* and the effect of Chlorophylliptum solution on their adhesion process.

Materials and methods. The study was carries out on 5 clinical isolates of *C. albicans* and 5 isolates of *C.glabrata* taken from patients of the Department of Thoracic Surgery and identified in the bacteriological laboratory of the Poltava Regional Clinical Hospital.

Adhesive potential of the studied clinical strains of microorganisms were evaluated by using the standard method of Brillis V.I. et al. Human erythrocytes O (I) of the Rh (+) group were used as a universal model for the studying adhesive properties [10]. For light microscopy, preparations were stained by Gram method.

Counting was performed on no less than 50 red blood cells, including no more than 5 red blood cells in a field of view. Adhesive properties were evaluated by the microbial adhesion index (MAI), i.e. the average number of microbial cells on a single erythrocyte, which is involved in the adhesive process. According to the findings obtained, microorganisms were divided into non-adhesive (MAI ≤ 1.75), low-adhesive (MAI = 1.76-2.5), medium-adhesive (MAI = 2.51 — 4.0) and high-adhesive (MAI ≥ 4.1).

We used an alcoholic solution of Chlorophylliptum thick extract as an active agent and 96% ethanol as an additive ("Halychpharm" corporation, Lviv, Ukraine, No. UA / 4551/02/01 dated October, 31, 2016). In order to determine the subfungistatic concentrations (SfC) of the preparation relative to the strains of microorganisms studied, we determined the fungistatic concentrations of the Chlorophylliptum extract, which were active towards the stains.

The working concentration of the medicine (subfungistatic concentrations) used in the course of our research was ¼ of fungistatic concentration of the studied extract for each type of pathogen. Thus, Chlorophylliptum extract SfC relative to *C.albicans* was 0,35 mg/ml, and relative to *C.glabrata* – 0,31 mg/ml.

The statistical analysis of the findings obtained was performed using the standard software packages "STATISTICA +" and "Microsoft Excel 2010".

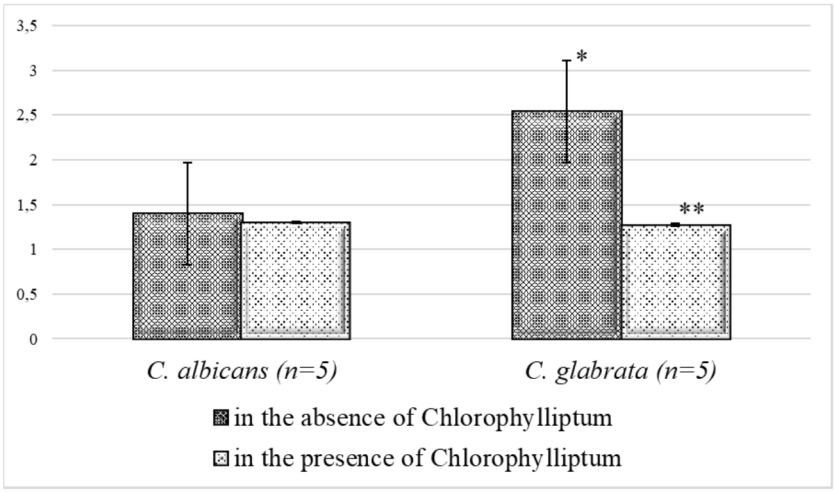

Fig.1 Microbial adhesion indices of *C.albicans* and *C.glabrata* clinical isolates, M ± m (* - reliability of differences in microbial adhesion indices between *C.glabrata* and *C.albicans*, p <0.05; ** - reliability of differences in microbial adhesion indices between *C.glabrata* and *C.albicans* in the presence of Chlorophylliptum taken in subfungistatic concentration, p <0.05).

Results of the study and their discussion. According to the results obtained, MAI of the clinical strains of *C.albicans* was 1.4 ± 0.22 that, according to the Brillis method, indicated their non-adhesiveness. However, this index for the isolates of the species *C.glabrata* exceeded *C.albicans* MAI in 1.8 times (p <0.05) and was found out within 2.54 ± 0.37 (fig. 1)).

This is quite logical, since, unlike other representatives of the genus *Candida, C. glabrata* does not form invasive hyphal structures that retain epithelial tissues and invade them. Instead of this, the *C. glabrata* species uses adhesion molecules to bind to the epithelial cells of the macroorganism.

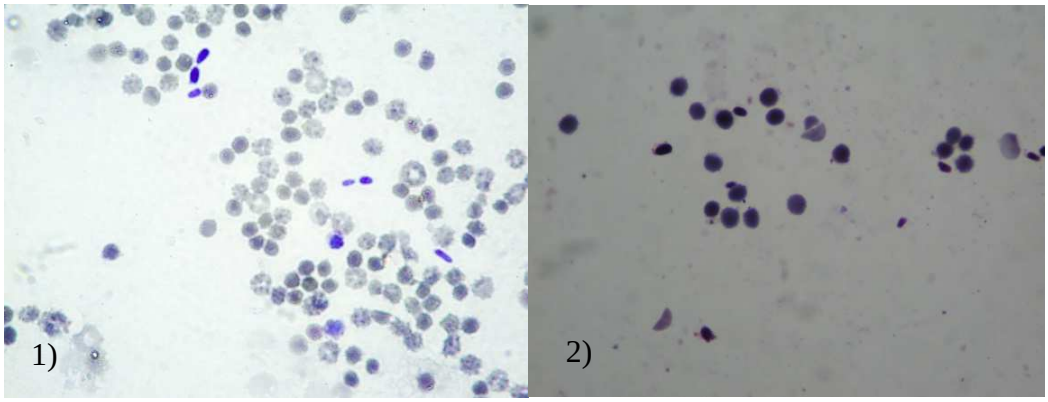

Fig. 2. Microscopic picture of *C.albicans* adhesion properties, stained by Gram method. Magnification: x100 lens; ocular x 10. 1) - without Chlorophylliptum extract; 2) - in the presence of Chlorophylliptum extract taken in subfungistatic concentrations).

It was found out that the SfC of alcohol solution of Chlorophylliptum extract did not affect the adhesive properties of clinical strains of *C.albicans*, as the microbial adhesion index of these pathogens in the presence of the medicine did not significantly change (fig. 2).

In turn, the Chlorophylliptum extract demonstrated considerable inhibitory effect on the ability of clinical isolates of *C. glabrata* to adhere to human erythrocytes. Microbial adhesion index of *Candida spp*. in the presence of SfC solution demonstrated two-fold drop ($p < 0.05$) in comparison with the microbial adhesion index of *C. glabrata* without Chlorophylliptum (fig. 3).

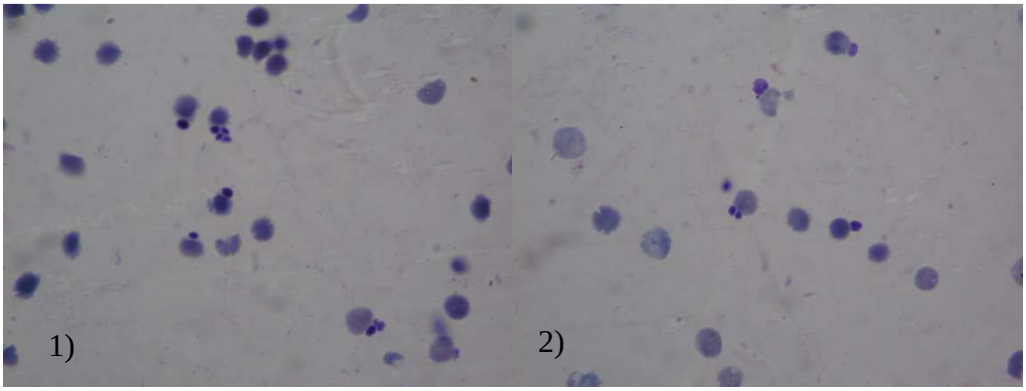

Fig. 3. Microscopic picture of *C. glabrata* adhesion properties, stained by Gram method. Magnification: x 100 lens; ocular x10.
1) - without Chlorophylliptum extract; 2) - in the presence of Chlorophylliptum extract taken in subfungistatic concentrations).

Candida spp. are ubiquitous commensals of humans, which can lead to life-threatening systemic infections. While almost all *Candida spp.* are relatively closely related members of the genus, *C. glabrata* is separated phylogenetically [5]. That's why they demonstrate differences in the persistence, infection strategies and susceptibility to antifungals [14]. For examples, in *C. glabrata*, the widely used azoles are intrinsically less active than in *C. albicans* [15].

Both *C. albicans* and *C. glabrata* have large protein families of adhesins. However, the Als proteins with theirAgglutinin-like sequences are predominant adhesins for *C. albicans.* Moreover, this member of *Candida spp.* produces hypha due to expression of hypha-associated adhesins Als3 and Hwp1 [15]. On the other hands, *C. glabrata* include approximately 20 genes, which encode production of adhesins. The Epa proteins provide attachment to epithelial cells and macrophages. Additionally, expression of non- Epa adhesins can mediate attachment of *C. glabrata* to other cell types [4].

Because of this, the results, we have got, demonstrate completely different adhesiveness of *C. glabrata* and *C. albicans.*

C. glabrata can serve as an etiological factor of diseases of the mucous membranes (oral cavity, oesophagus, vagina or urinary tract), as well as can cause severe, life-threatening invasive candidiasis by itself or can cause co-infections along with other *Candida* species such as *C. albicans* and *C. tropicalis*. Therefore, the sensitivity of these microorganisms, isolated from surgical patients, to Chlorophylliptum extract and the suppression of their adhesion properties under its influence state new opportunities and perspectives in the prevention and treatment of fungal postoperative complications.

Conclusion

The clinical isolates of *C. albicans* yield to the strains of *C. glabrata* by their adhesive properties. Subfungistatic concentrations of the alcoholic solution of Chlorophylliptum do not affect the adhesive properties of *C. albicans*, but reliably inhibit the ability of *C. glabrata* isolates to attach to the surface of human erythrocytes.

The study draws interest in further research of this drug as an alternative means to control candidiasis of different localization.

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Реферати

ВПЛИВ РОЗЧИНУ ХЛРОФІЛІПТУ НА АДГЕЗИВНІ ВЛАСТИВОСТІ CANDIDA SPP

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Метою даного дослідження було вивчення адгезивних властивостей клінічних штамів *C.albicans, C. glabrata* та ефекту розчину хлорофілліптута на процес адгезії. Дослідження проводилося на 5 клінічних ізолятах *C. albicans* та 5 ізолятах *C.glabrata*, отриманих від пацієнтів хірургічного відділення. Клінічний потенціал вивчених клінічних штамів мікроорганізмів оцінювали за допомогою стандартної методики Бріліс В.І. та ін. Ми використали спиротовий розчин екстракту хлорофілліпту як активної речовини та 96% етанолу в якості додаткової (корпорація "Галичфарм", Львів, Україна, № UA / 4551/02/01 від 31 жовтня 2016 р.). Субфунгістатичні концентрації, використані в ході дослідження, становили ¼ фунгістатичної концентрації вивченого екстракту для кожного типу збудника. Результати. В результаті досліджень встановлено, що клінічні штами *C. albicans* та *C.glabrata* володіли різною чутливістю до екстракту хлорофіліпту та здатністю до адгезії. Так, штами *C. albicans* виявилися неадгезивними, в той час як *C.glabrata* володіли середніми адгезивними властивостями. Різниця індексів їх адгезивності складала 1,8. Виявлено, що субфунгістатичні концентрації спиртового розчину екстракту хлорофілліпту не впливають на адгезивні властивості клінічних штамів *C.albicans.* У свою чергу, екстракт хлорофілліпту демонструє значний інгібуючий вплив на здатність клінічних ізолятів *C. glabrata* адгезуватися на еритроцитах людини. Клінічні штами *C. glabrata* володіють вищими адгезивними властивостями, порівняно з *C.albicans.* Спиртовий екстракт хлорофіліпту достовірно пригнічує здатність до адгезії *C. glabrata.*

Ключові слова: *C.albicans, C. glabrata,* адгезія, хлорофіліпт.

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ВЛИЯНИЕ РАСТВОРА ХЛОРОФИЛЛИПТА НА АДГЕЗИВНЫЕ СВОЙСТВА CANDIDA SPP

Ананьева М.Н., Фаустова М.А., Сизова Л.М., Рева Р.А.

Целью исследования было изучить адгезивные свойства клинических штаммов *C.albicans, C.glabrata* и влияние на них раствора хлорофиллипта. Исследование проводилось на 5 клинических изолятах *C. albicans* и 5 изолятах *C.glabrata*, выделенных у пациентов хирургического отделения. Адгезионный потенциал исследуемых клинических штаммов микроорганизмов оценивали с использованием стандартного метода Brillis V.I. и другие. Мы использовали спиртовой раствор густого экстракта хлорофиллипта в качестве активного агента и 96% этанола в качестве добавки (корпорация «Галичфарм», Львов, Украина, № UA / 4551/02/01 от 31 октября 2016 года). Субфунгистатические концентрации, используемые в ходе нашего исследования, составляли ¼ фунгистатической концентрации изученного экстракта для каждого типа патогена. Выяснилось, что субфунгистатические концентрации спиртового раствора экстракта хлорофиллипта не влияют на адгезивные свойства клинических штаммов *C.albicans*. В свою очередь экстракт хлорофиллипта продемонстрировал значительное ингибирующее действие на способность клинических изолятов *C. glabrata* адгезироваться на эритроцитах человека. Клинические штаммы C. glabrata обладают более высокими адгезивными свойствами по сравнению с C.albicans. Спиртовой экстракт хлорофиллипта достоверно подавляет способность к адгезии C. glabrata.

Ключевые слова: *C.albicans, C. glabrata,* адгезия, хлорофиллипт.

Рецензент Гаврилюк А.О.