Effect of static magnetic fields against some virulence factor of *C.albicans*: germ tube formation, adhesion ability and biofilm formation

أثر المجال المغناطيسي الثابت على بعض عوامل امراضية *Candida albicans*: تكوين انبوب الانبات ، التصاقها وقابليتها على تكوين الغشاء الحيوي

Nada S. Rezooqe
College of Science for Women / Baghdad University
ندى صباح رزوقي
كلية العلوم للبنات / جامعة بغداد

Abstract

In vitro,* inhibitory effects of static magnetic fields in the type of two poles and negative and positive pole on some virulence factors belong to *C.albicans* as pathogenic microorganism was investigated in this study. According to the findings of this study static magnetic fields in the type of two poles and negative pole displayed a variable degree of inhibitory effects against this microorganism *via* its virulence factors such as: germ tube formation, adhesion ability and biofilm formation in which negative pole showed more strongly effect than two poles as inhibitory values ranged from 35% as germination ability, 30% adhesion ability after 3hrs of treatment and 2.2mg/disk dry weight as biofilm formation after 72hrs of treatment compared with the inhibitory values for two poles ranged from 30% as germination ability, 25% adhesion ability after 3hrs of treatment and 2.7mg/ disk dry weight as biofilm formation after 72hrs of treatment . Following our earlier demonstration, the effects of positive pole also investigated in this study as 10 % for germination ability, 10% adhesion ability after 3hrs of treatment and 3mg/ disk dry weight as biofilm formation after 72hrs of treatment. These results revealed that static magnetic fields in the type of negative pole may be useful as antifungal agent against *Candida albicans.

المستخلص

اجريت هذه الدراسة خارج الجسم الحي للتحري عن الاثر التثبيطي للمجال المغناطيسي الثابت ضد بعض عوامل امراضية خميرة المبيضات *C.albicans* كاحدى الممرضات الشائعة . حيث بينت النتائج التي تم الحصول عليها ان المجال المغناطيسي الثابت بشكليه الثنائي القطب وذو القطب السالب قد اظهرا فعلا تثبيطيا ولكن بدرجات متفاوتة ضد الكائن قيد الدراسة بحيث القطب السالب كان فعله التثبيطي اكبر على الكائن قيد الدراسة مقارنة بالفعل التثبيطي للقطبين معا من خلال النتائج التي تم تسجيلها والتي كانت 35% كنسبة انبات و 30% نسبة التصاق بعد 3 ساعات من المعاملة و 2.2 مليغرام / للقرص كوزن جاف لتكوينها الغشاء الحيوي بعد 72 ساعة مقارنة للفعل التثبيطي للقطبين معا حيث كانت 30% كنسبة انبات , 25% نسبة التصاق بعد 3 ساعات من المعاملة و 2.7 مليغرام / للقرص كوزن جاف لتكوينها الغشاء الحيوي بعد 72 ساعة . في حين ان استخدام القطب الموجب اعطى النتائج الاتية والتي كانت 10% كنسبة انبات و 10% نسبة التصاق بعد 3 ساعات من المعاملة و 3 مليغرام / للقرص كوزن جاف لتكوينها الغشاء الحيوي بعد 72 ساعة . من خلال النتائج التي تم الحصول عليها نجد ان بالامكان استخدام المجال المغناطيسي الثابت بشكله كقطب سالب كعامل مثبط للفطريات لابادة هذه الخميرة الممرضة .

Introduction

The electromagnetic field (EMF) is composed of both an electric and a magnetic field. The electric field is due to the presence of charged particles (such as electrons) and the magnetic field is due to the movement of the charged particles (such as an

electron current) [1]. In modern life The magnetic and electromagnetic fields have been studied by several authors because Electricity represents one of the basic forms of energy and produces significant effects on biological tissues in both positive and negative ways [1,2].

Medical magnetic fields have been found in extensive research in Europe, in humans and all kinds of animal species, to have many positive actions in the body. The medical magnetic fields work by stimulating the acupuncture system, the immune system of the body, improving circulation and oxygen levels in tissues, relaxing muscles, stimulating tissue healing, healing fractures and strengthening bones faster, decreasing nerve irritability, removing swelling, decreasing clotting and improving cell metabolism. Some very strong medical magnetic fields can actually stimulate muscles and nerves -used for incontinence, rebuilding muscles, nerves and depression [2].

In recent decades , many scientific studies have demonstrated that electric magnetic fields of extremely low frequency can influence the biological systems describing these effects on ‘‘smaller’’ biological objects such as bacteria [3,4,5], yeast and fungi [4,6,7,8]. But there are no conclusive results describing these effects on pathogenic yeast such as *candida albicans* and lts virulence factor such as: germ tube formation and biofilm formation.

Candida albicans is an endogenous organism. It can be found in 40-80% of normal human beings. It is present in the mouth, gut, and vagina. It may be present as a commensal or as pathogenic organism. Infections with *Candida* usually occur when a patient has some alteration in cellular immunity, normal flora or normal physiology. Also Transplantation procedures, immunosuppression, the use of chronic indwelling devices, and prolonged intensive care unit stays have increased the prevalence of Candidiasis [9]. Forty percent of patients with microbial colonization of intravenous catheters develop fungemia, with consequences ranging from focal disease to severe sepsis and death [10, 11].

These clinical observations emphasize the importance of Candidiasis as infections caused by *Candida albicans*. So In this study, we tried to investigate a possibility of using static magnetic fields as antifungal agents against some virulence factor of *C.albicans* such as: germ tube formation, adhesion ability and biofilm formation.

Materials & Methods:

Test Organism

A clinical isolate of *Candida albicans* was used for this study, obtained from Biology Dep., College of Science for Women, Baghdad University. The yeast was maintained on Sabouraud Dextrose Agar, identified classically by using standard Microbiological methods [12].

Inoculum Preparation

The inoculum suspensions of *C.albicans* were obtained by taking five colonies from 24hrs old cultures grown on SDA. The colonies were suspended in 5ml of sterile saline (0.85% Nacl), shaked for 15 seconds and the density was adjusted with sterile saline according to McFarland standard of 0.5 ($\approx$1-5x10^6 Cfu/ml) [13].

Static magnetic fields

In order to study the effect of a static magnetic field on *Candida albicans*, first the magnets stimulation area was built Figure (1). Then, the cultures positioned inside this area as shown in Figure (2).

Fig. (1): Magnets stimulation area

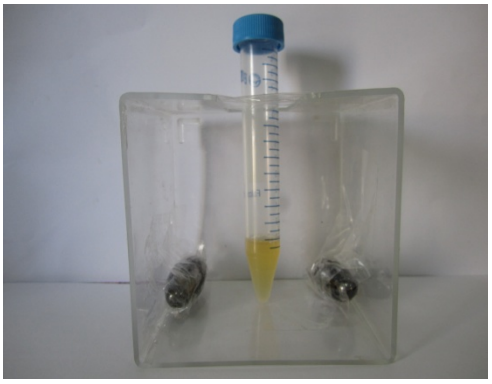

Fig. (2): *C.albicans* culture inside the magnets stimulation area.

Quantitative assessment of germ tube formation

To detected the effect of static magnetic field on germ tube formation by *C.albicans*, modified method was used [14] as follows: 0.2 ml of the yeast suspension treated with a static magnetic field at different times (0, 1, 2, 3 hrs) was added to a test tube containing 2 ml of human serum, mixed well and incubated at 35^{o}C for 3 hrs. Results were recorded by taking a drop of the suspension and examined under a microscope to confirm the presence or absence of germ tubes comparing with control.

Adhesion assays

Yeast adhesion to exfoliated buccal epithelial cells was determined by light microscopy as described by [15] with slight modification as follows: Human buccal epithelial cells (HBEC) were collected on cotton swabs from healthy female volunteers and transferred to phosphate-buffered saline (PBS) pH 7.2. HBEC were washed three times with PBS and counted by using a hemocytometer, One ml of the yeast suspension previously treated with a static magnetic field at different times (0, 1, 2, 3) hrs was added to a test tube containing 1 ml of HBEC at a concentration of $1x10^4$ cell/ml in PBS then incubated for 1h at 37C with shaking, after centrifugation three times at 5000 r.p.m for 10 min with PBS for washing, the sediment were suspended in PBS, one drop from this solution was transferred to a microscope slide and stained with crystal violet stain to determine the adherence. Adherence was calculated as a percentage of HBEC with adhering *Candida* cells comparing with control.

Biofilm formation assays

To detected the effect of static magnetic field on biofilm formation by the yeast cells, the modified method was used [16] as follows: inoculum of the yeast suspension from overnight cultures was added to silicone disk and then incubated at 37C for different times (0, 12, 24 and 72 hrs) under effect of a static magnetic field and incubated in fetal bovine serum (FBS) for 24 hrs at 37ºC. After this pretreatment, disks were washed with phosphate-buffered saline (PBS) to remove excess yeast cells then dry weight was estimated. All tests were done in triplicate.

Results and discussion:

According to the findings of these study static magnetic fields as the type of two poles and of negative pole displayed a variable degree of inhibitory effects against this microorganism *via* its virulence factors such as: germ tube formation, adhesion ability and biofilm formation as summarized in Figures (3, 4, 6). negative pole showed more strong effect than this of two poles where the inhibitory values ranged from 35% as germ tube formation ability, 30% adhesion ability after 3hrs of treatment and 2.2mg/disk dry weight as biofilm formation after 72hrs of treatment compared with the inhibitory values of the two poles ranged from 30% as germ tube formation ability, 25% adhesion ability after 3hrs of treatment and 2.7mg/ disk dry weight as biofilm formation after 72hrs of treatment.

Following our earlier demonstration, the effect of positive pole also mentioned in Figures (3,4, 6) ranging from 10 % as germ tube formation ability, 10% adhesion ability after 3hrs of treatment and 3mg/ disk dry weight as biofilm formation after 72hrs of treatment.

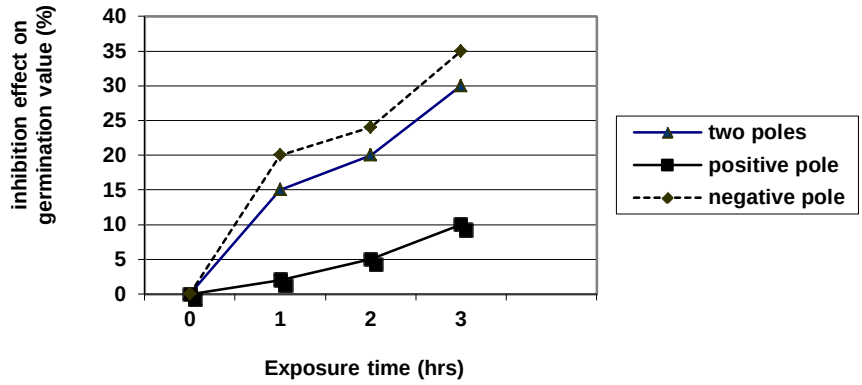

Fig (3): Inhibitory effect of static magnetic fields on germ tube formation by *C.albicans*

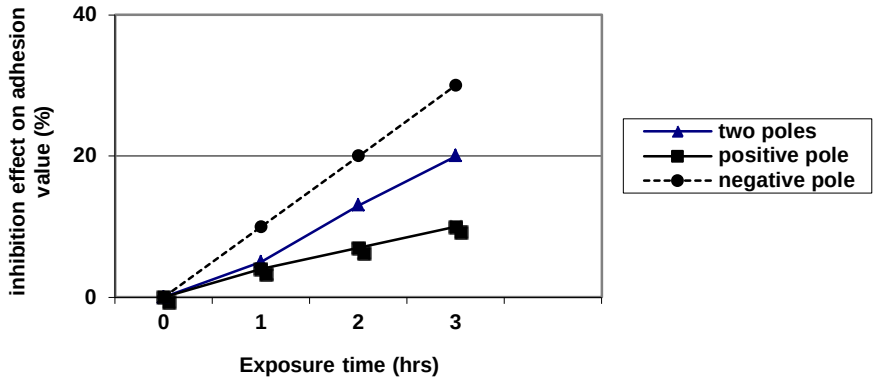

Fig (4) Inhibitory effect of static magnetic fields on adhesion ability of *C.albicans*

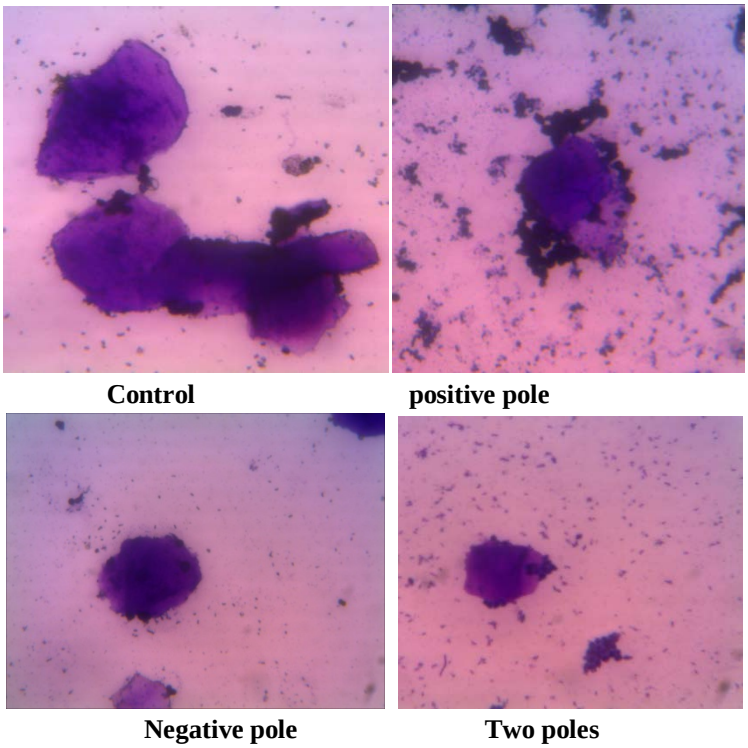

Fig (5): Adhesion ability of *C.albicans* after 3 hrs of treatment with static magnetic fields at 400X

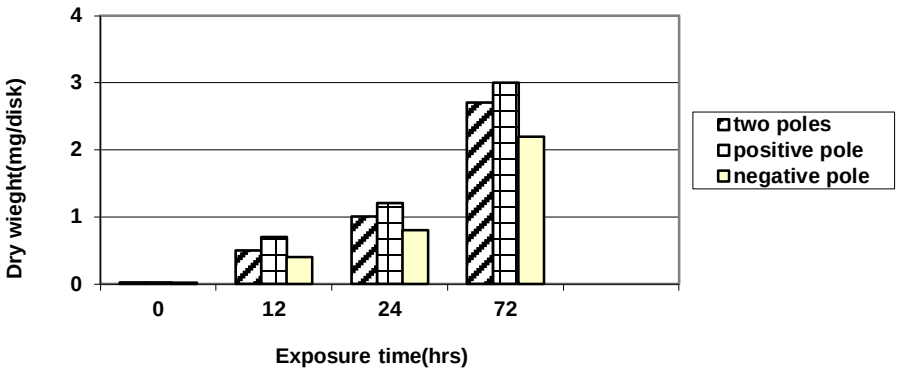

Fig (6): Effect of static magnetic fields on biofilm formation by *C.albicans*

The obtained results in this study suggested that static magnetic fields have an inhibitory effect on some virulence factor of *C.albicans* such as : germ tube formation and biofilm formation in different ratios according to the type of static magnetic fields used as two poles or that of negative pole (North) and the time of exposure , in contrary to the effect of positive pole (South) which has Stimulated effect with prolonged exposure, these results agree with others who reported that the stimulation or inhibition of microbial growth depend on the field strength, frequency and types of microorganism [3,4,6,17].

The mechanism of the magnetic field effect on yeast cells was not known, but the main theories that tried to explain the biological effects of electromagnetic fields are based on the possible effects on the permeability of the ionic channels in the membrane [18]. This can affect ion transport into the cells and this can result in biological changes in the organisms [6]. The other possible effects are the formation

of free radicals due to magnetic field exposure [3] in addition to other main effect is the influence of electromagnetic fields on carbohydrate metabolism [7,19,20]. [20] also reported differences in cDNAfingerprints between cells exposed to magnetic fields and unexposed cells, and showed that the magnetic field induced changes in gene expression. The gene expression in the exposed cells showed that the magnetic field induced different metabolic responses with regard to carbohydrate metabolism.

In the other hand, Cellular events in biological systems, such as cell aggregation, recognition of reporter molecules and cell migration are mediated by the specific interaction of cell surface molecules and the study of these interactions is essential to understand the chemical functions and recognition ability of how the magnetic field can effects on those interactions. In this study we found that the inhibitory effect of static magnetic fields on biofilm formation of *C.albicans* had different reactions according to type of static magnetic fields used and the strength of reaction increased with the time of exposure, which agrees with the study of [8] when they used a novel measurement method of the binding force between a micrometer-sized particle and a solid surface in an electrolyte solution which has been established by using the electromagnetophoretic buoyancy on the particle to investigate the binding force between a yeast cell surface and an oligosaccharide-binding protein fixed on a silica capillary wall and found satisfactory results.

Conclusions

In conclusion, the present study shows that the virulence of *C.albicans* could be suppressed by the static magnetics in different strengths depending on the type of static magnetic fields and the time of exposure, and this could be applied as new antifungal agents against Candidiasis.

References

1. Carozzi, T., Eriksson, A. and Lundborg, B. (2009). Electromagnetic Field Theory. 3rd edition. Uppsala, widen.
2. Frank, S. and Greenebaum, B. (2007). Biological and Medical Aspects of Electromagnetic Fields. 3rd edition.Taylor & Francis Group, LLC CRC Press - NW.
3. Fojta, L.b., Stras, L.k., Vladimı, A., Vetterla, B. and Mardac, J.S. (2004). Comparison of the low-frequency magnetic field effects on bacteria *Escherichia coli, Leclercia adecarboxylata* and *Staphylococcus aurous*. Bioelectrochemistry. 63 :337– 341
4. Gao, M., Zhang, J. and Feng1, H. (2011). Extremely Low Frequency Magnetic Field Effects on Metabolite of *Aspergillus niger*. Bioelectromagnetics. 32:73-78.
5. Strask, L., Vetterl, V. & Fojt, L. (2005). Effects of 50Hz Magnetic Fields on the Viability of Different Bacterial Strains. Electromagnetic Biology and Medicine. 24: 293–300.
6. Uzun, L., Necdet S., Safarikova, M., Safarik, I. and Denizli1, A. (2011). Copper Biosorption on Magnetically Modified Yeast Cells under Magnetic Field. Separation Science and Technology. 46: 1045–1051.
7. Galonja - Coghill, T. A., Kostadinovia, L. M. and Bojat, N. C. (2009). Magnetically altered Ethanol Fermentation Capacity of *Saccharomyces cerevisiae*. Proc. Nat. Sci, Matica Srpska Novi Sad. 117: 119—123.

8. Iiguni, Y. & Watarai, H. (2007). Electromagnetophoretic Force Measurement of a Single Binding Interaction between Lectin and Yeast Cell Surfaces. Analytical Sciences. 23: 121-126.

9. Mavar, A., Thewes, S. and Hube, B. (2005). Systemic fungal infections caused by *Candida* species: epidemiology, infection process and virulence attributes. Curr. Drug. Targets. 6:863-874.

10. Wey, S. B., Mori, M. A., Pfaller, R. F. and Wenzel, R. P. (1988). Hospital-acquired candidemia. The attributable mortality and excess length of stay. Arch. Intern. Med. 148:2642–2645.

11. Anaissie, E. J., Rex, J. H., Uzun, O. and Vartivarian, S. (1998). Predictors of adverse outcome in cancer patients with candidemia. Am. J. Med. 104:238–245.

12. Evans, E., G. and Richardson, M. D. (1989). Medical Mycology: a practical approach. Practical approach series. Oxford, England.

13. Boukraa, L., Benbarek, H. and Moussa, A. (2008). Synergistic Action of starch and Honey against *Candida albicans* in correlation with Diastase number. Brazilian J. Microbiology. 39: 40-43.

14. Al-Maomory, A.S. (2004). Activity of low power laser on candidiasis by using photosensitizers (*In vivo* & *In vitro*). M. SC. Thesis, College of Education (Ibn Al-Haitham). University of Baghdad.

15. Bailey A., wadsworth, Th, E. and Calderone, R. (1995). Adherence of *Candida albicans* to human Buccal Epithelial cells: Host-Induced Protein Synthesis and signaling Events. Infection & Immunity. 63(2): 569-572.

16. Chandra, J., Mukherjee, P. K., Leidich, S. D., Faddoul, F. F., L. L., Hoyer, L. J. and Ghannoum, M. A. (2001). Antifungal resistance of candidal biofilms formed on denture acrylic *in vitro*. J. Dent. Res. 80:903–908.

17. Ivanova, V., Hristov, J., Dobreva, E., Al-Hassan, Z. and Penchev, I. (1996). Performance of a magnetically stabilized bed reactor with immobilized yeast cells. Appl Biochem Biotechnol. 59:187–198.

18. Koch, B., Sommarin, M., Persson, B., Salford, L.G. and Eberhardt, J.L. (2003). Interaction between weak low frequency magnetic fields and cell membranes. Bioelectromagnetics. 24:395–402.

19. Nascimento, L.F., Botura, J.G. and Mota, R.P. (2003). Glucose consume and growth of *E. coli* under electromagnetic field. Rev Inst Med Trop Sao Paulo. 45:65–67.

20. Potenza, L., Ubaldi, L., Sanctis, R.D., Bellis, R.D., Cucchiarini, L. and Dacha,`M. (2004). Effects of a static magnetic field on cell growth and gene expression in *Escherichia coli*. Mutat Res Genet Toxicol Environ Mutagen. 561:53–62.