Postantifungal Effect of Natural and Synthetic antimycotic drugs in treatment of recurrent vaginal candidiasis

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Abstract: Twenty five isolates of vaginal *Candida albicans* were tested for Postantifungal effects (PAFE) of natural (latex sap) and synthetic antifungal agents (nystatin, amphotericin B, fluconazole, ketoconazole and 5-fluorocytosine). The natural latex sap antifungals exhibited the highest postantifungal effect (PAFE). A little or none postantifungal effect (PAFE was determined for fluconazole. A marginal PAFE postantifungal effect (PAFE) was observed for ketoconazole. The mean duration of PAFE of latex sap 1, latex sap 2, nystatin, amphotericin B, 5-fluorocytosine, ketoconazole and fluconazole were 3.5 h, 3.6 3, 3.2 h, 3.0 h, 2.8 h, 0.4 h and o.15 h respectively. The mean percentage reduction in adhesion of oral *Candida albicans* to vaginal epithelial cells during PAFE were 80%, 79%, 77%, 76%, 76%, 14% and 12% on exposure to latex sap 1, latex sap 2, nystatin, amphotericin B, 5-fluorocytosine, ketoconazole and fluconazole respectively.
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1. Introduction

Infections caused by *Candida albicans* are reported among the most common fungal infections affecting humans. Their incidence has greatly increased with widespread use of broad-spectrum antibiotics, antitumors and immunosuppressive drugs (Budtz, 1990 and Lyons and White, 2000). *Candida albicans* is the most common fungal pathogen isolated from the vaginal infection. It may be considered a vaginonophilic microorganism (Wahlin, 1991 and Odd, 1994). Vaginal candidosis is one of the most frequent diseases caused by *Candida albicans* (Cannon *et al.,* 1995). Although some authors claim that candidal colonization is usually associated with vaginal infections (Korting and Schaller 2001and Akpan and Morgan 2002).

The effect of antibiotics on bacterial growth has been extensively studied including the study of post antibiotic effect (PAE) that refers to suppression of bacterial growth persisted following limited exposure of bacteria to antimicrobial agents and subsequent removal of the antibiotic (Bernard *et al.,* 1994). There is a little data on postantifungal effect (PAFE) of antifungals on vaginal *Candida albicans* (Egusa *et al.,* 2000).

Adhesion is considered to be an important first step in colonization and in subsequent symptomatic and asymptomatic infection of vaginal mucosa (Barga *et al*., 1995). Adhesion of the pathogenic *Candida albicans* to epithelial cells has been inhibited by glycosidases (Darwazeh *et al.,* 1997 and Granger *et al.*, 2005).

The aim of the present work is to achieve maximum antifungal activity while simultaneously minimizing patient exposure to the drug to reduce the emergence of resistance through study role of PAFE of natural antifungal drug (latex sap) and synthetic antifungal drugs in therapy of vaginal infections through inhibition of *Candida albicans* adherence to BECs.

2. Materials and Methods

Yeast isolation

Twenty-five samples were collected from a typical case of vaginitis through 6 months. *Candida albicans* isolated on Sabouraud glucose agar (Difco, USA) for 24 h at 37^{o}C and were subculture weekly.

Morphological and biochemical characterization

All strains were subjected to morphological and biochemical tests for *Candida albicans* identification using API 20 C system (Brawner and Cutler, 1986).

Antifungal agents

Latex sap 1 (natural antifungal) was tapped from stem of articulated laticifers of *Lactuca sativus* (*Compositae*) and latex sap 2 were tapped from non-articulated laticifers of *Asclepias curassavica* (*Asclepiadaceae*) in Pharmacognosy Department, Faculty of Pharmacy, Zagazig University. Latex saps were stored at – 28 ^{o}C. After thawing, this latex was diluted in distilled water (1:17) containing sodium azide (Giordani and Noat, 1988). Five synthetic antifungal agents including nystatin, amphotericin B, fluconazole, ketoconazole and 5- fluorocytosine were used. Nystatin and amphotericin B (Sigma, USA) were dissolved in dimethylsulphoxide (DMSO) and absolute

ethanol (3:2 ratio). 5- fluorocytosine (Sigma) was dissolved in sterile distilled water. Ketoconazole (Janssen, Beerse, Belgium) was dissolved in DMSO. Fluconazole (Pfizer, Groton, CT, USA) was dissolved in absolute methanol. The five synthetic antifungals were prepared initially as 10000 µg /ml solution and stored at – 20 ^{o}C before use.

Determination of minimum inhibitory concentration (MIC)

As PAFE is estimated by exposure of the organisms to varying MIC of antimicrobial agents for short periods, the MIC was evaluated by methods of Craig and Gudmundsson (1996) using broth dilution technique with an initial inoculum of 1- 5 x 10^5 CFU/ml. MIC was defined as the lowest concentration of the drug inhibited the growth of yeast cells.

Determination of postantifungal effect (PAFE)

The method used for determination of postantifungal effect of bacteria was applied with minor modifications to evaluate PAFE of yeast (Craig and Gudmundsson, 1996). Yeast cells maintained on to Sabouraud's agar were inoculated on fresh plates and incubated overnight for 24 h at 37 ^{o}C. The organisms were harvested and a cell suspension was prepared in phosphate buffer saline (PBS) at 520 nm to an optical density 1.5. From this cell suspension, 0.5 ml was added to tubes containing 2 ml broth (control) and 2 ml broth/ drug solution (test) in which the drug concentrations varied from 4- 8 times the MIC. The drug concentrations used were sub-cidal concentrations of nystatin (6 MIC), amphotericin B (8 MIC), fluconazole (4 MIC), ketoconazole (4 MIC) and 5-fluorocytosine(8 MIC). This gave a cell suspension 10^6 -10^7 cells/ml in each assay tube. The tubes were incubated at 37 ^{o}C for 1 h in a rotary incubator. Following this limited exposure the drugs were removed by two cycles of centrifugation for 10 min at 3000 g. Afterwards the supernatant was completely decanted and the pellets were re-suspended in 2.5 ml PBS at pH 7.

To study PAFE, aliquots of 1 ml from each cell suspension was added to 2 ml Sabouraud's broth and incubated at 37 ^{o}C for 18 h. Growth of yeast cells and the change in turbidity was measured spectrophotometerically (absorbance 595 nm) at 30 min intervals for 18 h. The duration of PAFE was calculated using the formula of Lowdin *et al.,* (1993) PAFE = T – C, where T was the time required for relative optical density of drug- exposed cell suspension to reach the 0.05 absorbance level after removal of the drug and C was the drug free control cell suspension to reach the same absorbance level**.** Thus T – C expressed the time in which the antifungal agent capable of causing growth suppression of the organism following limited exposure to the drug (PAFE).

Adhesion assay

Epithelial cells

The urine samples were obtained in the morning from healthy women then centrifuged at 350 rpm for 10 min to harvest the epithelial cells. The sediment was washed twice in phosphate buffered saline (PBS) pH 7.2 and the number of cells were estimated microscopy with a counting chamber, then standerdized to 10 ^{5} cell/ml in PBS buffer.

Adhesion assay

A mixture of equal volumes of epithelial cells (10 ^{5} cell/ml) and *Candida albicans* (10 ^{8} cell/ml) was incubated in plastic tubes on a rotator at 37 ºC for 2 h. The epithelial-yeast mixture was passed through polycarbonate filters (12 µm pore size) to remove non adhering yeast. Adhesion was evaluated spectrophotometer by measuring the optical density of epithelial cells with adherent yeasts according to according to Jones and Fowler (1994).

3. Results

Minimum inhibitory concentration (MIC)

The MICs of latex sap 1, latex sap 2, nystatin, amphotericin B, fluconazole, ketoconazole and 5-fluorocytosine against the all tested *Candida albicans* isolates were given in Table 1.

Postantifungal effect (PAFE)

PAFE of different antifungal agents on *Candida albicans* isolates after exposure for 1 h to 4- 8 times MIC were shown in Table 2 and Figure 1. Significant PAFE were induced in all *Candida albicans* isolates by natural antifungals (latex sap 1 and latex sap 2) and synthetic antifungals (nystatin, amphotericin B and 5-fluorocytosine). However, little or non PAFE was observed for ketoconazole and fluconazole. Natural antifungals (latex sap 1 and latex sap 2) exhibited higher PAFE than synthetic antifungals. Figure 2 showed growth suppression of ***C1*** following removal of latex sap 1, latex sap 2, nystatin, amphotericin B and 5- fluorocytosine comparable to the unexposed controls. In contrast, there was immediately normal growth after ketoconazole and fluconazole removal. Similar results were obtained for the other tested *Candida albicans* isolates.

Effect of natural and synthetic antifungals on adhesion of *Candida albicans* to epithelial cells

Table 3 revealed that Candida albicans isolates when grown on medium containing natural antifungals latex sap exhibited higher percentage reduction of adhesion to epithelial cells than when grown on medium containing synthetic antifungals comparable to the untreated control. The mean percentage reduction of fungal adhesion during PAFE were 80%, 79%, 77%, 76%, 76%, 14% and 12% on exposure to latex sap 1, latex sap 2, nystatin, amphotericin B, 5- fluorocytosine, ketoconazole and fluconazole respectively.

Table 1. Minimum inhibitory concentration (MIC)

Isolate	MIC (µg/ml)						
	Latex sap 1	Latex sap 2	Nystatin	Amphotericin B	Fluorocytosine	Ketoconazole	Flucona zole
C1	0.11	0.12	2.61	0.34	0.15	12.52	25
C2	0.12	0.22	2.51	0.30	0.15	12.51	25
C3	0.10	0.13	1.25	0.33	0.15	12.52	25
C4	0.14	0.24	1.25	0.15	0.31	12.50	50
C5	0.20	0.23	1.25	0.15	0.32	6.25	12.5
C6	0.21	0.12	2.51	0.15	0.33	6.25	12.5
C7	0.23	0.32	2.51	0.31	0.15	12.51	50
C8	0.23	0.21	1.25	0.32	0.15	12.52	25
C9	0.14	0.11	1.25	0.31	0.15	12.53	25
C10	0.13	0.22	1.25	0.15	0.15	12.52	25
C11	0.1 1	0.13	2.51	0.15	0.15	252	50
C12	0.21	0.24	2.50	0.15	0.15	6.25	12.5
C13	0.21	0.33	1.25	0.15	0.3**2**	6.25	12.5
C14	0.22	0.22	1.25	0.15	0.15	12.51	50
C15	0.14	0.11	1.25	0.15	0.15	12.52	25
C16	0.13	0.21	2.50	0.33	0.15	12.53	25
C17	0.24	0.22	2.51	0.33	0.15	12.52	25
C18	0.22	0.13	1.25	0.31	0.15	6.25	12.5
C19	0.22	0.23	1.25	0.15	0.15	6.25	12.5
C20	0.21	0.32	1.25	0.15	0.31	12.51	50
C21	0.13	0.13	1.25	0.15	0.15	12.52	25
C22	0.11	0.11	1.25	0.31	0.15	12.53	25
C23	0.11	0.22	1.25	0.32	0.15	12.51	25
C24	0.21	0.23	2.52	0.31	0.15	12.53	25
C25	0.20	0.31	2.51	0.15	0.15	12.53	25

Table 2. Postantifungal effect (PAFE)

Isolate	PAFE (hours)						
	Latex sap 1	Latex sap 2	Nystatin	Amphotericin B	Fluorocytosine	ketoconazole	Fluconazole
C1	5.23	4.23	4.50	3.22	2.81	0.32	0.11
C2	3.4**3**	3.31	3.31	2.51	2.62	0.42	0.14
C3	3.52	3.82	3.81	2.51	3.51	0.51	0.10
C4	4.21	3.61	2.62	3.22	4.22	0.51	0.12
C5	3.31	4.23	2.43	3.32	3.31	0.61	0.21
C6	3.82	3.32	3.71	2.81	3.22	0.42	0.20
C7	3.63	3.71	3.4**2**	2.61	2.51	0.33	0.20
C8	3.52	3.43	3.53	3.52	2.52	0.63	0.21
C9	3.71	3.51	4.22	4.22	3.22	0.43	0.12
C10	3.4**1**	4.21	3.32	3.31	2.31	0.42	0.13
C11	3.52	3.31	3.53	2.81	3.51	0.32	0.12
C12	4.23	3.83	3.53	2.61	3.22	0.61	0.21
C13	3.32	3.62	4.24	3.22	3.31	0.41	0.21
C14	3.81	3.41	3.32	3.33	2.81	0.31	0.22
C15	3.62	5.23	3.84	2.53	3.21	0.41	0.13
C16	3.40	3.4**1**	2.63	2.54	2.82	0.51	0.14
C17	5.22	3.53	4.22	2.53	2.61	0.52	0.11
C18	3.4**1**	3.24	3.33	2.52	3.21	0.62	0.14
C19	3.52	3.40	3.32	2.53	2.32	0.42	0.10
C20	4.23	3.53	3.81	3.44	2.02	0.31	0.12
C21	3.34	3.22	3.51	3.53	2.51	0.42	0.14
C22	3.82	3.30	4.21	4.23	2.52	0.52	0.10
C23	3.61	2.80	3.31	3.34	2.01	0.61	0.12
C24	4.22	3.61	3.23	2.82	2.51	0.41	0.11
C25	4.31	3.53	2.64	3.21	2.4**2**	0.31	0.10
Mean	**3.8**	**3.5**	**3.3**	**3**.0	**2.8**	**0.4**	**0.15**

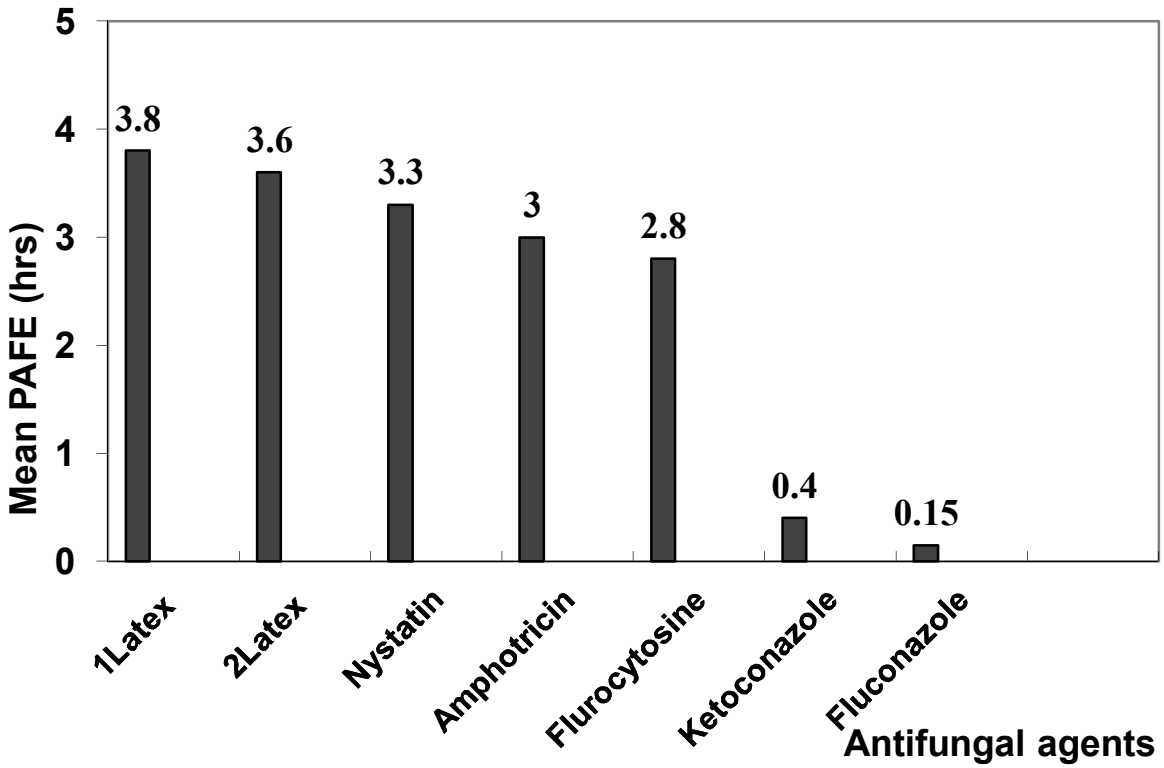

Figure 1. Postantifungal effects of different antifungal agents

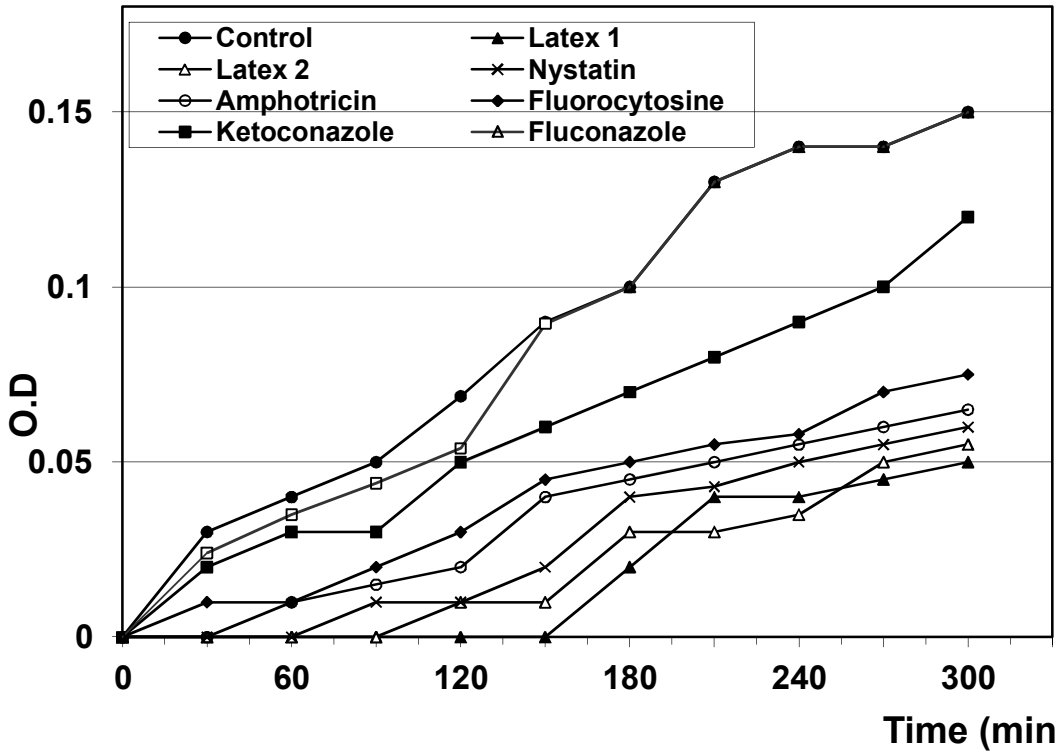

Figure 2. Growth curves of *Candida albicans (C1)* after limited exposure to different antifungal agents

Table 3 Percentage reduction of *Candida albicans* adhesion to epithelial cells with natural and synthetic antifungals

Isolate	Percentage reduction of adhesion (%)						
	Latex sap 1	Latex sap 2	Nystatin	Amphotericin B	Fluorocytosine	ketocona zole	Flucona zole
C1	85	81	80	78	75	20	10
C2	83	77	78	77	80	20	12
C3	80	78	77	78	78	18	15
C4	75	79	78	75	77	16	10
C5	81	80	75	80	78	15	12
C6	77	75	80	78	75	10	15
C7	78	81	78	77	70	12	10
C8	85	77	77	78	77	15	10
C9	80	78	78	75	78	10	10
C10	75	75	75	70	75	16	11
C11	81	81	70	77	70	15	12
C12	77	77	73	78	75	10	10
C13	78	78	76	75	80	12	10
C14	85	79	80	70	78	15	13
C15	79	80	78	75	78	10	14
C16	84	75	77	80	77	12	10
C17	80	81	78	78	78	15	10
C18	75	77	75	77	75	10	11
C19	81	78	80	78	70	16	12
C20	77	77	78	75	73	15	10
C21	78	78	77	70	77	10	10
C22	85	79	78	77	78	12	13
C23	79	80	75	78	75	15	14
C24	84	75	70	75	70	10	10
C25	77	78	73	70	73	11	10
Mean	**80**	**79**	**77**	**76**	**76**	**14**	**12**

4. Discussion

Candida albicans is considered the most common yeast isolated from vagina of either healthy or medically compromised women (Akpan and Morgan, 2002). Their presence does not usually result in disease unless there are predisposing factors such as diabetes (Lamey *et al*., 1988 and Peterson, 1992), malegnancy (Jobbins *et al.,* 1992), cytotoxic therapy (Heimdahl *et al*., 1990) or human immunodeficiency virus infection (Beighton *et al*., 1990, Blair *et al.,* 1995). Other risk factors of oral candidiasis include extremities of age, pregnancy (Noverr and Huffnagle, 2004).

Effective synthetic antifungal agents such as nystatin, amphotericin B, fluconazole, ketoconazole and 5- fluorocytosine and natural antifungals latex sap obtained from articulated laticifers of *Lactuca sativus* and non-articulated laticifers of *Asclepias curassavica* are currently available for treatment of vaginal-candidiasis (Menon *et al*., 2001). The efficacy of treatment is dependent on many factors including the diluent effect of vaginal wash that tend to reduce the availability of the drug below the effective therapeutic concentration (Bader *et al*., 2001). In general, the goal with respect to treatment with antifungal agents is to maintain the drug concentration above the MIC for almost the entire dosing period (Willis *et al*., 2001).

In our study the MIC values of latex sap, nystatin, amphotericin B and 5- fluorocytosine were much lower than that of ketoconazole and fluconazole. Similar results have been reported by Tacconelli *et al*., (2002). The results revealed that limited exposure to latex sap, nystatin, amphotricin B and 5-fluorocytosine elicited high PAFE in tested *Candida albicans* strains. Similar results for amphotericin B and 5- fluorocytosine were reported by Anil *et al*., (2001).

Cell wall degradation reflecting a fungal cell wall disorganization seems to be the result of hydrolysis of glycosidic linkages by specific glycosidases (Spadari *et al*., 1998). A major focus of interest in these studies has been the identification of the fungal cell wall components involved in attachment process. Continuous series of studies found that chitin which is a macromolecule present in the cell walls of almost all fungi was involved in the adhesion of the fungus to mammalian tissues (Wightman *et al,*. 2004). Milan *et al.* (2001) found that differing growth conditions altered the cell surface of *Candida albicans* resulting in modified adhesion ability of the fungus. It can be thus assumed that the fungal cell wall components are involved in the adhesion of *Candida albicans*. The adhesion results indicated that limited exposure of *Candida*

albicans strains to latex sap, nystatin, amphotericin B and 5- fluorocytosine significantly reduced their ability to adhere when compared to with that of fluconazole and ketoconazole. Adhesion of the pathogenic fungus *Candida albicans* to epithelial cells has been the subject of a number of studies (Barga *et al.,* 1996 and Sundstrom *et al.,* 2002).

The PAFE and reduced adhesion may be related to the mechanism of action of these antifungal agents. Latex sap act specifically on fungal cell walls by activating glycosidase activity exhibited highest PAFE and reduction in adhesion of *Candida albicans* to epithelial cells. The polyenes alter the permeability of the fungal cytoplasmic membrane by binding to ergosterol and fungistrol (Willis *et al.,* 2001). The DNA- analogue, 5- fluorocytosine, is transported to fungal cell and converted to 5-fluorouracil which is incorporated into DNA in place of uracil resulting in inhibition of protein and DNA synthesis (Wu *et al.,* 1996 and Oh *et al.,* 2005). The azoles cause alteration in fungal cell membranes by blocking 14-demethylation step in ergosterol biosynthesis and this mode of action is reversible (Munez *et al.,* 2001) explaining much lower PAFE of azoles than those of polyenes and 5- fluorocytosine in our results. The minimum growth suppression of the azoles is due to resistant of *Candida albicans* strains to these drugs as implied by high MIC values.

On conclusion, it is possible to achieve maximum antifungal activity while simultaneously minimizing patient exposure to the drug to reduce the emergence of resistance. Our results showed that the concept of PAFE should not be considered only in terms of fungal growth but also as impacting on microbial virulence factors such as adhesion and glycosidase production. latex sap (natural antifungal) can be used in therapy of *Candida albicans* vaginal infections because of the highest PAFE and through the increase of glycosidase activity that degrade fungal cell wall causing inhibition of *Candida albicans* adhesion.

References

1. Akpan, A. and Morgan, R. (2002): Vaginal candidiasis. Postgrad. Med. J. 78: 455 – 459.
2. Anil, S., Elleopola, A. and Samaranayake, L. (2001): Post-antifungal effect on polyenes, azoles and DNA- analogue agents against vaginal *Candida albicans*. J. Oral Pathol. 30: 481 – 488.
3. Badr O., Klein, S. and Hube B. (2001): Virulence factors of oral *Candida albicans*. Mol. Microbiol: 6: 1431- 1444.
4. Barga, P., Maci, S., Sasso, M. and Bohn, M. (1995): Experimental evidence for a role of rilopirox, nystatin and fluconazole on adherence of *Candida albicans*. Chemotherapy 42 (4): 259-65.
5. Barga, P., Sasso, M., Maci, S and Bohn, M. (1996): Inhibition of *Candida albicans* adherence to human epithelial cells. Arzneimittelforschung 45 (1): 84-7.
6. Beighton, D., Hellyer, P. and Heath, M. (1990): Vaginal candidiasis. Arch. Oral Biol.35: 173- 5.
7. Blair, Y., Bagg, J., and Chestnutt, I. (1995): *Candida albicans* serotype and adherence to epithelial cells. Epidemiol. 23: 100- 3.
8. Brenard, C., Valentin, A. and Reynes, J (1994): Relationship between fluconazole sensitivity of *Candida albicans* serotype and adherence. Eur. J. Clin. Microbiol. Infect. Dis. 13: 711 – 716.
9. Budtz, E. (1990): Etiology, pathogensis, therapy and prophylaxis of vaginal yeast infections. Acta Odontol Scand 48: 61-69
10. Cannon, R., Masson, A. and Monk, B. (1995): Clearance, colonization, or candidosis. J. Dent.Res. 74: 1152- 61.
11. Craig, W. and Gudmundsson, S. (1991): The post-antibiotic effect. Williams and Wilkin 403-431.
12. Darwazeh, A., Farlane, T. and Lamey, P. (1997): The in- vitro adhesion of *Candida albicans* to epithelial cells. J. Oral Pathol. Med 26: 233- 36.
13. Egusa, H., Nikawa, H. and Samaranayake, L. (2000): The post-antibiotic effect of polyenes on *Candida albicans*. J. Pathol. Med 29: 206- 213.
14. Granger, L., Flemiken, M. and Cutler, E. (2005): Yeast wall proteins of *Candida albicans*. Microbiology 151 (5); 1631- 1644.
15. Heimdahl, A. and Nord, C. (1990): Vaginal yeast infections in immunocompromized and seriously diseased patients. Acta Odontol. Scand. 48: 77-84.
16. Jobbins, J., Parson, K. and Bagg, J. (1992): Vaginal carriage of yeast in patients with advanced malignant disease. J.Oral Pathol. Med. 21: 305- 8.
17. Jones,D. and Fowler, S. (1994): The adherence of to human epithelial cells. International J. of Pharmaceutics 105: 71- 75.
18. Korting, H. and Schaller, M. (2001): New developments in medical mycology. Hautarzt, 2: 91 – 97.
19. Lamey, P., Darwaza, A. and Fisher, B. (1988): Secretor status, candidal carriage and candidal infection in patients with diabetes. J. Pathol. 17: 354 –7.
20. Lowdin, E., Odenholt, T. and Bengtsson, S. (1993): A new method in determine post-antifungal effect. Antimicrobal Agents Chemotherapy 37: 2200- 2205.

21. Lyons C.and White, T. (2000): Transcriptional analysis of antifungal drug resistance in *Candida albicans*. Antimicrob. Agent Chemother. 44 (9): 2296- 303.
22. Menon, T., Amamaheswari, K. and Solomon, S. (2001): Efficacy of fluconazole in treatment of candidiasis. Acta. Trop. 22: 151- 154.
23. Milan, E., Costa, P. and Matta, D. (2001): Colonization of *Candida albicans*. Mycoses 44: 173 – 277.
24. Munez, M., Balboa, J. and Mana, P. (2002): Effect of psychological stress and alprazolam on development of candidosis. Clin. Diagn. Lab. Immun. 852 – 857.
25. Noveer, M. and Huffnagle, G. (2004): Regulation of *Candida albicans* metabolism. Infect. Immun. 72 (11) 6202- 6210.
26. Odd, F. (1994): *Candida albicans,* the life and times of pathogenic yeast. J. Med. Vet. Mycol. 32: 1-8.
27. Oh, S., Chang G. and Zhao, X. (2005): *Candida albicans* proteins. Microbiology 151 (3); 673-681.
28. Peterson, D. (1992): Candidosis. Clin. Geriatr. Med. 8: 513- 27.
29. Romagnoli, G., Nisini, R., Chiani, P. and Teloni, R. (2004): Effect of nystatin and fluconazole on adherence of *Candida albicans.* J. Cell Biol. 75 (1): 117- 125.
30. Spadari, E., Arosio, M., and Artini, M. (1998): *Candida albicans* morphotypes and in-vitro adhesivity to cells of the human vaginal epithelium in HIV positive and AIDS patients. Minerva Stomato 47 (7): 293-7.
31. Sundstrom, P., Balish, E. and Allen, C. (2002): Essential role of *Candida albicans* hyphal wall protein in lethal candidiasis. J. Infect. Dis. 521 – 530.
32. Tacconelli, E., Posteraro, B. and Fadda, C. (2002): Azole susceptibility patterns in oral *Candida albicans*. Acquir. Immun. Defice Syndr. 1:38 44.
33. Wenzl, T., Horchen, H. and Skopnik, H. (1998): Pharmacokinetics of fluconazole in premature infants. Eur. J. Pediatr. 157 (8): 661- 2.
34. Wightman, R., Bates, S. and Sudbery, P. (2004): *Candida albicans* adhesion to mammalian tissues. J. Cell Biol. 164 (4): 581 – 591.
35. Willis, A., Coulter, W. and Hayes, J. (2001): The influence of antifungal agents on virulence of *Candida albicans.* Sur. Oral. Med. Oral Pathol. 3: 317- 321.
36. Wu, T., Samaranayake, L., Cao, B. and Wang, J.(1996): In- vitro proteins production by *Candida albicans* from individual with and without HIV. J. Med. Microbiol. 44 (4): 311- 6.

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