

Research Article

Antimicrobial Actions of Antihypertensives

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ABSTRACT

The increased incidence of hypertension and diabetes mellitus worldwide puts the human health under peril. Susceptibility of these patients for various infections endangers their life. Alarming increasing antibacterial resistance is a cause of major concern. By the year 2015 chances of people dying due to drug resistant bacterial infections will be more than that from cancer. Hence there is a need for the development of potent newer antimicrobials or to test and confirm the antibacterial properties of non-antibacterial drugs used to treat various conditions. Hypertension is one area where the antihypertensive drugs used to treat the condition have proved their potential activity against various infections. The antihypertensive drugs which were observed to have antimicrobial action are calcium channel blockers like verapamil, amlodipine, nifedipine and lacidipine, central sympatholytic drug alpha methyl dopa and beta blocker like propranolol. Verapamil which is an ion channel blocker has shown potential against mycobacterium tuberculosis when used along with approved anti tubercular drugs and helped in prevention of drug resistance. Similarly, alpha methyl dopa showed significant in vitro activity against atypical mycobacteria. Propranolol exhibited anti fungal and antitoxoplasmal activity. Verapamil helped to reverse chloroquine resistance developed by plasmodium falcifarum malaria parasite. This suggests that properties of these antihypertensive in treating various bacterial and nonbacterial infections should be studied extensively by many clinical studies to authenticate their use.

Keywords: antimicrobial, antihypertensive, bacterial, nonbacterial.

INTRODUCTION

Incidence of hypertension as a disease complex is rising alarmingly worldwide due to increased longevity and as a result of faulty and stressful life style. Diabetes mellitus is one of the predisposing cause for the onset of hypertension which is known to enhance the susceptibility of patients to various infections. This requires the need for selection of proper antimicrobial drugs. Some studies have reported that by year 2050, more and more individuals will die of drug resistant bacterial infections than that from cancer^{1,2}. Recently various organisms have developed resistance against existing antimicrobial drugs which warrants the need to discover newer antimicrobial drugs or to explore the possible antimicrobial action of available non-antimicrobial drugs^{3,4}. Co-existence of hypertension and diabetes and increased chances of infection in these class of patients necessitates the need to know whether any of the drugs used for this condition possess any antimicrobial action. Certain antihypertensive drugs were found to have antimicrobial actions. These are some of the calcium channel blockers, central sympatholytic like alpha methyl dopa and beta blocker like propranolol⁵⁻¹⁰.

Calcium channel blockers

Verapamil- Verapamil is a calcium channel blocker of voltage dependent L- type calcium channels. It is an ion channel blocker. Recently ion channel blockers including

verapamil have been tried for their activity against mycobacterium tuberculosis [M.tuberculosis] bacilli.

M. tuberculosis bacillus due to its impenetrable cell wall, its intracellular survival in host cell and its long duplication time makes it difficult to control pathogen and hence it can become resistant to antitubercular drugs. This requires long term chemotherapy to eradicate this infection, which can create an obstacle to the drug adherence and for the patient's compliance. Prolonged drug therapy also exposes the patient for drug toxicity. This necessitates the need for better and time efficient drugs or the use of adjuvant drugs which will potentiate the effectiveness of existing drugs. Ion channel blocker verapamil was tested against drug resistant M.tuberculosis strain both in human infected macrophages and in vitro. This drug being an ion channel blocker is also an efflux inhibitor. In vitro studies, it exhibited synergistic inhibitory activity against tubercular organisms when it was combined with antiTB drugs like Isoniazide and Rifampicin^{5,11,12}.

It is observed that M.tuberculosis efflux genes get overexpressed when they are exposed to antibiotics in vitro and also within the macrophages. This efflux pump activity contributes to drug resistance by making the intracellular drug concentration sub inhibitory¹³⁻¹⁶. This also exposes the organism to develop drug resistant mutants¹⁵⁻¹⁸. Verapamil showed rapid and high killing

activity against *M.tuberculosis* which correlates with decreased intracellular ATP levels suggesting the interference in the energy metabolism. Inhibition of respiratory chain complex, and decreased ATP production which provides energy for bacterial efflux pump mechanism which forms the major cause for drug resistance, gets inhibited by verapamil resulting in to the reduction of antiTB drug resistance. Thus, verapamil contributes to bacteriocidal effect¹⁹⁻²¹.

The ion channel blocker verapamil promotes vacuolar acidification by controlling the conditions in the phagosome vacuole needed for effective microbial killing and digestion through lysosomal hydrolytic enzymes^{22,23}. This is controlled through the transport of cations by eukaryotic vacuolar efflux forms. This is also coupled to the energy provided by electron transport chain enzyme which are located in the membrane and proton channels^{22,23}. Increased intracellular concentration of the drug enhances the transcription of the proton pump V-ATPase. This results in to enhanced phagosomal acidification²⁴ and increase in Ca²⁺ concentration in the lumen²⁵. This activates acidification dependent lysosomal hydrolases²⁴ and enhances macrophage mediated killing of the mycobacteria^{26,27}.

Prokaryotic and eukaryotic efflux pumps use the energy of either ATP [primary transporter] or PMF [secondary transporter] required for the extrusion of ions, noxious compounds or metabolites. Energy provided for this process is through oxidative phosphorylation^{21,28}. The energy production in both eukaryotic and prokaryotic cells starts with NADH dehydrogenase and ends up at the respective F1 F0 ATP synthase which is the key electron transport chain enzyme and is thought to be inhibited by ion channel blocker like verapamil either directly or indirectly^{21,28,29}.

Enhancement of the killing activity of macrophage by ion channel blocker like verapamil was considered to be dependent upon the availability of intracellular potassium and calcium^{26,27,30}. Gupta et al demonstrated that increase in calcium from intracellular calcium stores in macrophages as a result of L-type calcium channel inhibition led to reduction in mycobacterial population. *M.tuberculosis* modulates levels and activity of important intracellular second messengers to evade protective immune responses. Release of calcium from voltage gated calcium channels is responsible for regulation of immune responses to pathogens. Inhibition of these channels in infected macrophages induced the calcium influx, which was found to upregulate the expression of pro inflammatory genes, resulting in to the killing of intracellular mycobacteria and decreased mycobacterial population²⁵.

Oral verapamil is well tolerated with fewer side effects. Thus verapamil targets mycobacterium and host macrophages resulting in to - After entering in to the mycobacteria it initiates cascade of events resulting in to the inhibition of respiratory chain complexes and energy production required for the bacterial efflux activity which helps in the reduction of antibiotic resistance and potentiation of its activity. Verapamil promotes

phagosome acidification and enhances transcription of hydrolases in the host cell resulting in to inhibition of bacterial growth when combined with antitubercular drugs. This combination enhances mycobacterial killing, prevents drug resistance and shortens the duration of chemotherapy.

Verapamil was found to reverse chloroquine resistance of falciparum malaria organisms by inhibiting the efflux of the drug and by enhancing intraparasitic concentration of chloroquine from subinhibitory to therapeutic one^{31,32}.

Amlodipine

Amlodipine was found to be effective against various bacteria in decreasing order of sensitivity against staph.aureus, vibrio cholera, vibrio parahemolyticus, shigella spp, salmonella spp, bacillus spp. Amlodipine was bacteriocidal in nature both in vitro and vivo when studied in white mice. Amlodipine was found to be more effective than felodipine, lacidipine and nifedipine and was powerful antibacterial against large number of bacteria⁶. Study done with derivatives of phenothiazines revealed their in-vitro antimicrobial activity which was found to be closely linked to the halogen groups present in the basic tricyclic ring structure of phenothiazines. In amlodipine structure one benzene ring is attached to another which may be considered as an incomplete phenothiazine ring. The presence of halogen [chlorine] moiety may be responsible for antimicrobial activity of this compound³³.

Nifedipine

Nifedipine was found to have in vitro and in vivo antibacterial activity against 331 strains of bacteria out of which 3 were of Gram positive and 12 were of Gram negative group organisms. Observed MIC of nifedipine was between 25-200mcgms/ml against almost all organisms in vitro studies. It has bacteriostatic action. Hence nifedipine has proved as an antibacterial agent which can be considered for clinical use and for further studies to confirm and evaluate its antibacterial potential⁷.

Lacidipine

Lacidipine is a third-generation L type calcium channel blocker. It was screened for antibacterial activity against 389 Gram positive and negative bacterial strains. 233 bacterial strains failed to grow at concentration 50-200mcgm/ml of the drug. Their sensitivity pattern in descending order was as follows-staph. aureus, vibrio cholera, salmonella typhi, shigella, vibrio cholera, e.coli, bacillus spp, klebsiella and pseudomonas spp. Doses used in the animal study were non toxic and were well tolerated. Lacidipine was found to have inhibitory activity against v.cholerae 569 B in one study. It reduced the viability of the organisms and also the production of cholera toxin in animal model of rabbit ileum. Lacidipine was found to be bacteriostatic in nature⁸. Heat labile enterotoxin of e.coli is structurally and functionally similar to cholera toxin³⁴. Earlier studies have shown that enterotoxin of e.coli were found to be highly sensitive for lacidipine³⁵. Thus, lacidipine is both antibacterial and antitoxic also.

Centrally acting sympatholytic drug-Alpha methyl dopa

Alpha methyl dopa has shown significant in vitro activity against atypical mycobacteria like *M.avium* complex, *M.scrofulaceum*, *M.xenopi*, *M.marinum* and *M.fortuitum*.

Study done by N K Datta was extended to total of 53 strains of mycobacteria which also included 34 clinical isolates of drug sensitive and drug resistant strains of mycobacteria. For most of bacteria MIC was between 10-25 mcgms/ml⁹.

Beta blockers-propranolol

Propranolol was found to have anti fungal activity which involves inhibition of fungal hyphal growth by interfering cAMP-EFG 1 pathway¹⁰. Formation of hyphae plays an important role in the fungal virulence and tissue invasion³⁶. *Candida albicans* is an opportunistic pathogen which changes from non virulent yeast form to virulent hyphae form in response to the various environmental signals³⁷⁻³⁹. Addition of propranolol inhibited hyphae formation of the *C.albicans* by inhibiting the expression of agglutinin like sequence 3[ALS 3]and ALS 8 mRNA which are regulated by cAMP-EFG 1 pathway in *C.albicans*. It did not affect MAP-mitogen activated protein-kinase related expression of CST 20, HST 7 or CPH 1 mRNA. Hyphae transformation is induced by activation of the MAP kinase cascade and cAMP pathway which are controlled by RAAS protein⁴⁰⁻⁴². EFG 1 is a transcriptional factor^{43,44}. Hyphae specific protein such as ALS 3 and ALS 8 are cell wall proteins in *C.albicans* and their expressions are regulated by EFG1^{45,46}.

Although propranolol is used as a beta blocker in clinical medicine, the recommended blood level [50-100ng/ml]⁴⁷for the treatment greatly differs from the concentration required for the inhibition of hyphae formation. Thus propranolol might not be useful for the treatment of *C.albicans* infection. However it is expected that some propranolol derivative would effectively control the expression of EFG 1 and hyphae transformation of *C.albicans*.

Propranolol was found to inhibit *C.albicans* adherence on bio film formation on biotic and abiotic surfaces. *C.albicans* is associated with the nosocomial infection partly due to its ability to adhere to variety of biomaterial such as catheters and biofilms. These biofilms are related to the resistance to the antimicrobial agents and immune defences⁴⁸. Hyphal formation is essential for the structural integrity of the *C.albicans* biofilms⁴⁹. Propranolol probably binds to phosphatidic acid[PA], blocking diglycerol[DAG] synthesis⁵⁰. Propranolol acts mainly in the early stages of *C.albicans* biofilm formation. The attachment step required for the formation of biofilm is affected by propranolol. Thus inhibition of adherence to biotic surface and inhibition of germ tube formation may explain the effect of propranolol on *C.albicans* bio film formation. Adhesion of fungal cells to the epithelium was significantly affected by propranolol. Thus propranolol could prevent the early stages of *C.albicans* colonization and bio film formation on biological and non biological surfaces.

Adherence of microorganisms to cell surfaces involves numerous factors such as cell hydrophobicity, electrostatic forces and specific adhesions⁵¹. Inhibitory effect of propranolol on *C.albicans* biofilm formation may be associated with the effect on the expression of adhesion genes like agglutinin like sequence [ALS] genes or

adhesion like glycosyl phosphatidylinositol [GPI] linked cell surface proteins.

Propranolol inhibits germ tube formation by repressing the cAMP-EFG 1 pathway of *C.albicans*⁵². Moreover, propranolol inhibits morphogenesis by sequestering PA⁵⁰. This suggests that the transcription factor EFG 1 can be regulated by PA and/or DAG signaling pathway. Consequently the expression of EFG 1 target genes [ALS1, ALS 3] and GPI linked cell surface protein HWP1 is also repressed by propranolol. Thus inhibition of *C.albicans* adherence to cell surfaces and hyphal development by propranolol results in abnormal biofilms.

Effect of propranolol on toxoplasma infection-Potential of antitoxoplasma activity of propranolol was tested in acute and chronic phases of infection. Propranolol is a membrane stabilising agent which inhibits the entry of toxoplasma gondi trophozoite in to the cells. Efficiency of propranolol was increased against toxoplasma when combined with pyrimethamine⁵³.

CONCLUSION

Anti hypertensives like calcium channel blockers, central sympatholytic and beta blockers have been proved to have antimicrobial activities. Rising incidence of drug resistance in the area of various bacterial, mycobacterial, fungal and parasitic infection has raised the concern and challenged the survival of human race. The rate of discovery of newer antimicrobials is disproportionately slow as compared to the speed of development of antimicrobial drug resistance. Hence the need arises to tap the potential of non-antimicrobial drugs used to treat various conditions in which signs of infection become high due to underlying oxidative stress or due to associated conditions like hypertension. The additional antimicrobial action of antihypertensive drugs needs extensive studies both in vitro and in vivo. Minimum dose required to elicit antimicrobial action without causing any adverse effects has to be confirmed before using these drugs for associated infection in hypertensive patients.

REFERENCES

1. Antibiotic Resistance Threats in the United States, 2013, <http://www.cdc.gov/drugresistance/threat-report-2013/> accessed on November 21 2015.
2. Jirka T, Hafner M, Yerushalmi E, Smith R, Bellasio J, Vardavas R et al. Estimating the economic costs of antimicrobial resistance: Model and Results. Santa Monica, CA: RAND Corporation, 2014. http://www.rand.org/pubs/research_reports/RR911.html.
3. Anonymous. Dictionary of natural products on DVD. Chapman and Hall/CRC, London.
4. Oldfield E, Lin FY. Terpene biosynthesis: modularly rules. *Agnew Chem Int Ed Engl* 2012; 51: 1124-1137.
5. Gupta S, Tyagi S, Almeida DV, Maiga MC, Ammerman NC, Bishai WR. Acceleration of tuberculosis treatment by adjunctive therapy with verapamil as an efflux inhibitor. *Am J Respir Crit Care Med*. 2013;188:600-7.

6. Kumar KA, Ganguly K, Mazumdar K, Dutta NK, Dastidar SG, Chakrabarty AN. Amlodipine: a cardiovascular drug with powerful antimicrobial property. *Acta Microbiol Pol.* 2003;52:285-92.
7. Tapas P, Kumar D, Kaushiki M, Asish D, Jeyaseeli L, Sujata GD. Assessment of antibacterial activity of the cardiovascular drug nifedipine. *Oriental Pharmacy and Experimental Medicine* 2006; 6: 126-133.
8. Dasgupta A, Dastidar SG. Antibacterial & antitoxic effects of the cardiovascular drug lacidipine in an animal model. *The Indian Journal of Medical Research.* 2012;135:913-916.
9. Joydeep Mazumder, Devender Pathak, Rachna Kumria. Antacid Studies of Newly Developed Polyherbal Formulation. *International Journal of Drug Delivery Technology.* 2016; 6(1):27-29.
10. Dutta NK, mazumdar K, Dastidar S, Chakrabarty A, Shirataki Y, Motohashi N. In Vitro and In Vivo Antimycobacterial Activity of an Antihypertensive Agent Methyl-L-DOPA. *In vivo (Athens, Greece)* 2000; 19:539-45.
11. Ueno Y, Maruyama N, Kanno M, Watanabe T, Ogasawara A, Mikami T, et al. Effect of propranolol on hyphae formation signal in *Candida albicans*. *Biol Pharm Bull.* 2009; 32(1):129-31.
12. Pascal M, Guglielame P, Rossi E, Zara F, Riccardi G. mmpL7 Gene of *Mycobacterium tuberculosis* Is Responsible for Isoniazid Efflux in *Mycobacterium smegmatis*. *Antimicrob. Agents Chemother.* 2005; 49:4775-4777.
13. Adams KN, Szumowski JD, Ramakrishnan L. Verapamil, and its metabolite norverapamil, inhibit macrophage-induced, bacterial efflux pump-mediated tolerance to multiple anti-tubercular drugs. *J Infect Dis.* 2014; 210: 456-66.
14. Mitchison D, Davies G. The chemotherapy of tuberculosis: past, present and future. *Int J Tuberc Lung Dis.* 2012; 16(6):724-32.
15. Rayasam GV, Balganeshts TS. Exploring the potential of adjunct therapy in tuberculosis. *Trends Pharmacol Sci.* 2015; 36(8):506-13.
16. Machado D, Couto I, Perdigão J, Rodrigues L, Portugal I, Baptista P, et al. Contribution of Efflux to the Emergence of Isoniazid and Multidrug Resistance in *Mycobacterium tuberculosis*. *PLoS ONE* 2012; 7(4): e34538.
17. Schmalstieg AM, Srivastava S, Belkaya S, Deshpande D, Meek C, Leff R, et al. The antibiotic resistance arrow of time: efflux pump induction is a general first step in the evolution of mycobacterial drug resistance. *Antimicrob Agents Chemother.* 2012; 56: 4806-15.
18. Louw GE, Warren RM, Gey van Pittius NC, McEvoy CR, Van Helden PD, Victor TC. A balancing act: efflux/influx in mycobacterial drug resistance. *Antimicrob Agents Chemother.* 2009; 53: 3181-3189.
19. Gumbo T. Biological variability and the emergence of multidrug-resistant tuberculosis. *Nat Genet.* 2013; 45: 720-721.
20. Warman AJ, Rito TS, Fisher NE, Moss DM, Berry NG, O'Neill PM, et al. Antitubercular pharmacodynamics of phenothiazines. *J Antimicrob Chemother.* 2013; 68: 869-880.
21. de Kneegt GJ, ten Kate MT, van Soolingen D, Aarnoutse R, Boeree MJ, Bakker-Woudenberg IA, et al. Enhancement of in vitro activity of tuberculosis drugs by addition of thioridazine is not reflected by improved in vivo therapeutic efficacy. *Tuberculosis (Edinb).* 2014; 94: 701-707.
22. Blacka P, Warrena R, Louwa G, van Helden P, Victora T, Kanab B. Energy Metabolism and Drug Efflux in *Mycobacterium tuberculosis*. *Antimicrob. Agents Chemother.* 2014; 58: 2491-2503.
23. Hurst JK. What really happens in the neutrophil phagosome? *Free Radic Biol Med.* 2012; 53: 508-520.
24. Nauseef WM. How human neutrophils kill and degrade microbes: an integrated view. *Immunol Rev.* 2007; 219: 88-102.
25. Bruns H, Stegelmann F, Fabri M, Döhner K, van Zandbergen G, Wagner M, et al. Abelson tyrosine kinase controls phagosomal acidification required for killing of *Mycobacterium tuberculosis* in human macrophages. *J Immunol.* 2012; 189: 4069-4078.
26. Gupta S, Salam N, Srivastava V, Singla R, Behera D, Khayyam KU, et al. Voltage Gated Calcium Channels Negatively Regulate Protective Immunity to *Mycobacterium tuberculosis*. *PLoS ONE* 2009; 4: e5305.
27. Amaral L, Martins M, Viveiros M. Enhanced killing of intracellular multidrug-resistant *Mycobacterium tuberculosis* by compounds that affect the activity of efflux pumps. *J Antimicrob Chemother.* 2007; 59: 1237-1246.
28. Martins M, Viveiros M, Couto I, Amaral L. Targeting human macrophages for enhanced killing of intracellular XDR-TB and MDR-TB. *Int J Tuberc Lung Dis.* 2009; 13: 569-573.
29. Sazanov L. A giant molecular proton pump: structure and mechanism of respiratory complex I. *Nature Reviews Molecular Cell Biology* 2015; 16: 375-388.
30. Weinstein EA, Yano T, Li LS, Avarbock D, Avarbock A, Helm D, et al. Inhibitors of type II NADH:menaquinone oxidoreductase represent a class of antitubercular drugs. *Proc Natl Acad Sci USA.* 2005; 102: 4548-4553.
31. Viveiros M, Martins M, Rodrigues L, Machado D, Couto I, Ainsa J, et al. Inhibitors of mycobacterial efflux pumps as potential boosters for anti-tubercular drugs. *Expert Rev Anti Infect Ther.* 2012; 10(9): 983-998.
32. Martin SK, Oduola AM, Milhous WK. Reversal of chloroquine resistance in *Plasmodium falciparum* by verapamil. *Science.* 1987; 235: 899-901.
33. Martiney JA, Cerami A, Slater AF. Verapamil reversal of chloroquine resistance in the malaria parasite *Plasmodium falciparum* is specific for resistant parasites and independent of the weak base effect. *J Biol Chem.* 1995; 270: 22393-22398.
34. Annadurai S, Basu S, Ray S, Dastidar SG, Chakrabarty AN. Antibacterial activity of the antiinflammatory

- agent diclofenac sodium. *Indian J Exp Biol.* 1998; 36(1):86-90.
35. Tsuji T, Inoue T, Miyama A, Noda M. Glutamic acid-112 of the A subunit of heat-labile enterotoxin from enterotoxigenic *Escherichia coli* is important for ADP-ribosyl transferase activity. *FEBS Letters* 1991; 291: 319-321.
 36. Dasgupta A, Jeyaseeli L, Dutta NK, Mazumdar K, Karak P, Dastidar S et al. Studies on the Antimicrobial Potential of the Cardiovascular Drug Lacidipine. *In vivo* 2007; 21: 847-850.
 37. Brown AJ, Gow NA. Regulatory networks controlling *Candida albicans* morphogenesis. *Trends Microbiol.* 1999; 7: 333 – 338.
 38. Bailey DA, Feldmann PJ, Bovey M, Gow NA, Brown AJ. The *Candida albicans* HYR1 gene, which is activated in response to hyphal development, belongs to a gene family encoding yeast cell wall proteins. *J Bacteriol.* 1996;178:5353-5360.
 39. de Groot PW, de Boer AD, Cunningham J, Dekker HL, de Jong L, Hellingwerf KJ, et al. Proteomic analysis of *Candida albicans* cell walls reveals covalently bound carbohydrate-active enzymes and adhesins. *Eukaryot Cell.* 2004; 3: 955-965.
 40. Noverr MC, Huffnagle GB. Regulation of *Candida albicans* morphogenesis by fatty acid metabolites. *Infect Immun.* 2004; 72: 6206-6210.
 41. Schröppel K, Spröber K, Whiteway M, Thomas D, Röllinghoff M, Csank C. Repression of Hyphal Proteinase Expression by the Mitogen-Activated Protein (MAP) Kinase Phosphatase Cpp1p of *Candida albicans* Is Independent of the MAP Kinase Cek1p. *Infect. Immun.* December 2000; 68:7159-7161.
 42. Cloutier M, Castilla R, Bolduc N, Zelada A, Martineau P, Bouillon M, et al. The two isoforms of the cAMP-dependent protein kinase catalytic subunit are involved in the control of dimorphism in the human fungal pathogen *Candida albicans*. *Fungal Genet Biol.* 2003; 38: 133-141.
 43. Chen J, Zhou S, Wang Q, Chen X, Pan T, Liu H. Crk1, a novel Cdc2-related protein kinase, is required for hyphal development and virulence in *Candida albicans*. *Mol Cell Biol.* 2000; 20: 8696-8708.
 44. Bachewich C, Thomas DY, Whiteway M. Depletion of a Polo-like Kinase in *Candida albicans* Activates Cyclase-dependent Hyphal-like Growth. Koshland D, ed. *Molecular Biology of the Cell.* 2003; 14: 2163-2180.
 45. Rademacher F, Kehren V, Stoldt VR, Ernst JF. A *Candida albicans* chaperonin subunit (CaCct8p) as a suppressor of morphogenesis and Ras phenotypes in *C. albicans* and *Saccharomyces cerevisiae*. *Microbiology.* 1998; 144: 2951-2960.
 46. Zhao X, Oh SH, Cheng G, Green CB, Nuessen JA, Yeater K, et al. ALS3 and ALS8 represent a single locus that encodes a *Candida albicans* adhesin; functional comparisons between Als3p and Als1p. *Microbiology.* 2004; 150: 2415-2428.
 47. Stoldt VR, Sonneborn A, Leuker CE, Ernst JF. Efg1p, an essential regulator of morphogenesis of the human pathogen *Candida albicans*, is a member of a conserved class of bHLH proteins regulating morphogenetic processes in fungi. *EMBO J.* 1997; 16: 1982-1991.
 48. Ahnoff M, Ervik M, Lagerström PO, Persson BA, Vessman J. Drug level monitoring: cardiovascular drugs. *J Chromatogr.* 1985;340:73-138.
 49. Ramage G, Vandewalle K, Wickes BL, López-Ribot JL. Characteristics of biofilm formation by *Candida albicans*. *Rev Iberoam Micol.* 2001; 18:163-70.
 50. Chandra J, Kuhn DM, Mukherjee PK, Hoyer LL, McCormick T, Ghannoum MA. Biofilm formation by the fungal pathogen *Candida albicans*: development, architecture, and drug resistance. *J Bacteriol.* 2001; 183: 5385-5394.
 51. Baker CA, Desrosiers K, Dolan JW. Propranolol inhibits hyphal development in *Candida albicans*. *Antimicrob Agents Chemother.* 2002; 46: 3617-3620.
 52. Ramage G, Saville SP, Thomas DP, López-Ribot JL. *Candida* biofilms: an update. *Eukaryot Cell.* 2005; 4: 633-638.
 53. Lda SD, Pereira AL, Andrade AC, Kyaw CM, Silva-Pereira I
Propranolol inhibits *Candida albicans* adherence and biofilm formation on biotic and abiotic surfaces. *Int J Antimicrob Agents.* 2009; 34: 619-621.
 54. Montazeri M, Ebrahimzadeh MA, Ahmadpour E, Sharif M, Sarvi S, Daryani A. Evaluation of Propranolol Effect on Experimental Acute and Chronic Toxoplasmosis Using Quantitative PCR. *Antimicrob Agents Chemother.* 2016; 60: 7128-7133.