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Research Article

Effect of Fluconazole, An Anti-Fungal Drug, On Human Flora Bacteria

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Abstract

Fluconazole is an antifungal drug, which is in the class of azole derivatives and it is widely used worldwide. To date, there have been some studies published in the literature on the genotoxicity and cytotoxicity of fluconazole, indicating that the drug has a serious toxic effect. According to our research, there is no study on the antibacterial effect of fluconazole. Therefore, this research aimed to investigate the effect of fluconazole on the growth of some flora bacterial strains. The Mueller Hinton liquid medium containing different concentrations of fluconazole (24, 12, 6, and 3 mM) was prepared and inoculated with bacterial suspensions to adjust to Macfarland 0.5. After the culture was incubated for 24 hours at 150 rpm at 37°C, the growth of the strains was examined using the UV-visible spectrophotometer and CFU (colony forming unit) count. In addition, the growth of strains was repeated using sterile discs containing concentrations of fluconazole (24, 12, 6, and 3 mM) on the Mueller Hinton solid medium. After treated with the 24mM concentration of fluconazole, the growth rate of *Enterococcus faecalis, Enterobacter cloacae, Escherichia coli* and *Proteus mirabilis* strains, were reduced about 35%, 20%, 20% and 22%, respectively. Any of the drug concentration did not showed inhibitory effect on the bacterial strains on the Mueller Hinton solid medium.

Keywords: Fluconazole; Antifungal; Flora; Bacteria; Colony

Introduction

Fluconazole, a bis-triazole derivative, was synthesized in 1981 by replacing its imidazole nucleus [1]. The drug is a relatively small and water-soluble molecule compared to ketoconazole and itraconazole [2]. Fluconazole is widely used worldwide as an antifungal drug, especially in our country Turkey [3].

Oral dosage of the drug is in the form of tablets of 50, 100, 150 and 200 mg and its daily dose intake is 50-400 mg. In the treatment of candidemia, the maximum dose for *Candida* species is 400 mg daily, but for *Candida glabrata* is 800 mg. Increasing doses could be tolerated by patients, but increased hepatic and other side effects as the dose increased [4]. Side effects reported about the fluconazole are often associated with their interaction with the gastrointestinal tract. Serious side effects such as abdominal pain, diarrhea, flatulence, nausea, vomiting, leukopenia, thrombocytopenia and hyperlipidemia have been reported [5]. Somchit et al. [6] demonstrated the dose and time-dependent toxic effect of the drug on hepatocyte cells (Somchit et al. 2002). Balance of population is as important as the variety of bacterial strains in human flora [7]. The balanced condition between the host and intestinal microbiota is called symbiosis [8]. Microbiota can undergo changes by factors such as age, diet habits, lifestyle and genetic predisposition [9]. The amount and frequency of use of drugs can permanently change the microbiome [10]. Today, it is accepted that dysbiosis can cause many diseases in human [11]. Therefore, the flora bacteria are important. The number of pathogens increases in the impaired intestine; toxic substances accumulate as a result of the lack of useful flora to detoxify. Microorganisms have important functions in maintaining a healthy person's life, and sometimes disorders in the variety and balance of bacteria in the intestine can lead to the dominance of other pathogenic bacteria [12]. The family *Enterobacteriaceae* consists of many species and strains colonized in the small and large intestine, and also contains pathogenic members of the non-pathogenic commensal microbiota [13]. From *Enterobacteriaceae, Escherichia coli, Enterobacter cloacae, Proteus mirabilis, Proteus vulgaris* are the most frequently isolated strains from human intestins [14]. Most enterococcus species are normal flora of the gastrointestinal tract of humans. *Enterococcus fecalis* is one of enterococcus species. Therefore, this study aimed to analyze the effect of fluconazole on the *Escherichia coli, Enterococcus fecalis, Proteus mirabilis* and *Enterobacter cloacae*.

Materials and Methods

Reagents

The lab grade of fluconazole was purchased from Sigma-Aldrich (PHR1160-1G) and used as a test substance. The molecular weight of fluconazole (FCZ) was 306.27 g/ml and its chemical structure was as shown below.

Effect of fluconazole on bacterial strains by broth dilution method

Mueller Hinton broth medium containing different concentrations of fluconazole (serial dilutions made by creating a two-fold dilution with a starting concentration of 24 mM) were prepared and inoculated with an overnight culture of *Escherichia coli* (ATCC-25922), *Enterococcus fecalis* (ATCC-29212), *Proteus mirabilis* (ATCC7002) and *Enterobacter cloacae* (ATCC 13047) followed by adjusting to standard turbidity of 0.5 McFarland. After that, the cultures were incubated at 37°C at 150 rpm for overnight

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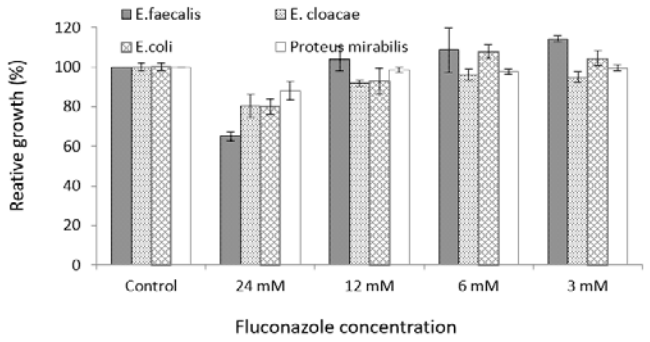

Figure 1: Relative growth of the *bacterial strains* in broth medium containing various concentrations of fluconazole. Control samples of *Escherichia coli, Enterococcus faecalis, Enterobacter cloacae* and *Proteus mirabilis* that were considered as 100% contained 7.6735E+14 CFU/ml, 5.5745E+21 CFU/ml, 2.72E+21 CFU/ml and 2.037E+22 CFU/ml respectively. All of the tests were conducted as three repeats.

to determine MIC (minimum inhibitory concentration) using UV-Visible spectrophotometry (Optizen 2120 UV) and CFU (colony forming units) counting.

Effect of fluconazole on bacterial strains by disc diffusion method

The bacterial strains of *Escherichia coli* (ATCC-25922), *Enterococcus fecalis* (ATCC-29212), *Proteus mirabilis* (ATCC7002) and *Enterobacter cloacae* (ATCC 13047) were cultured on Mueller Hinton agar plats by spread plate technique. After that sterile blank discs containing 24, 12, 6, and 3 mM of fluconazole were placed on the culture before incubation. Then the cultures were incubated at 37˚C for an overnight and the antibacterial effect of the fluconazole tried to assay by the zone diameter formed around the colonies [15].

Result

The maximum concentration of fluconazole (24 mM) had an inhibitory effect on the bacterial strains. After treated with the 24 mM concentration of fluconazole, the growth rate of *Enterococcus faecalis, Enterobacter cloacae, Escherichia coli* and *Proteus mirabilis* strains, were reduced about 35%, 20%, 20% and 22%, respectively. As it was shown in the (Figure 1), the inhibitory effect on the growth of bacteria is concentration dependent manner. In addition, as shown in Figure 2, not any zone was observed around the sterile disks containing fluconazole concentrations (24, 12, 6 and 3 mM) on the Mueller Hinton solid medium.

Discussion

Since human intestinal microbiota affects a lot of vital physiological processes, any change in microbial composition (dysbiosis) has a direct impact on human health [16]. In this research, drug concentration was calculated considering the concentration of fuconazole in the small intestine (105 ± 72 mL and 54 ± 41 mL) after maximum oral dose intake [17]. When fuconazole is given to patients orally (50, 100, 200 and 400 mg), it is directly contact the microorganism population in the gastrointestinal tract for a long time. After treated with the maximum concentration of fuconazole (24 mM) the growth rate of *Enterococcus faecalis, Enterobacter cloacae, Escherichia coli* and *Proteus mirabilis* strains, were reduced about 35%, 20%, 20% and 22%, respectively. The inhibitory effect

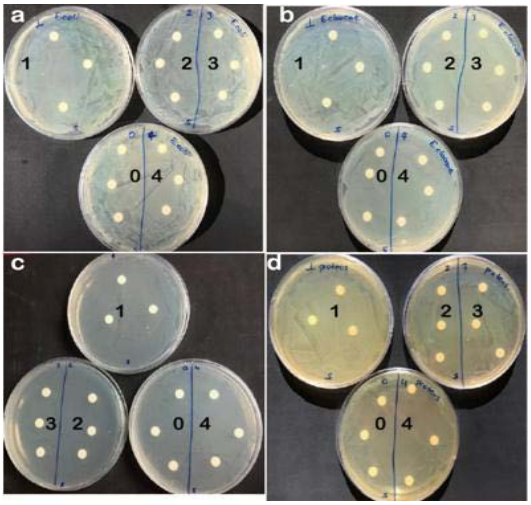

Figure 2: Growth of *Escherichia coli* (a), *Enterobacter cloacae* (b), *Enterococcus faecalis* (c) and *Proteus mirabilis* (d) on Mueller Hinton agar using sterile blank discs. Concentrations of fluconazole of the discs present in the plate-halves labeled by 1, 2, 3 and 4 contained 24, 12, 6 and 3 mM of fluconazole, respectively. Plate-halves labeled by zero did not contain fluconazole and it was control sample.

on the growth of the bacteria is less common as we move from high concentration to low concentrations. Before, a study on fish showed that a diet supplemented with *E. faecalis* significantly increased protease and lipase activities compared to a control feed. *E. faecalis* supplement significantly increased the production of propionic and butyric acid in the intestine [18]. In addition, decrease of *Escherichia coli* strain in the intestinal of the patients with ulcerative colitis cause to increase in the amount of non-normal flora bacteria even pathogenic strains in the intestinal tract [19]. Because colicin proteins expressed by *E. coli* inhibit bacterial growth of other species [20]. In a study on patients with ulcerative colitis, a reduced amount of *E. coli* bacteria was observed in the intestinal microflora, which was associated with joint involvement in patients with ulcerative colitis [21]. Patients with ulcerative colitis using fluconazole should consider this adverse effect, since culture concentrations of *E. coli* treated with fluconazole are reduced. The increase in the growth of *E. cloacae* in humans has been associated with obesity [22, 23]. *In vitro* studies have shown that in adipocytes induced by flagellin, they can increase hepatic fat accumulation by increasing lipolysis and glycerol synthesis from adipocytes [24, 25].

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