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Original Article

In Vitro Antifungal Effects of Duloxetine in Combination with Fluconazole on Fluconazole-Resistant *Candida Albicans*

Running Title: Effects of Duloxetine on Fluconazole-Resistant *Candida*

Ehsan Momeni[1]*, Abbas Alimoradian[2], Mojtaba Didehdar[3], Ali Tajic[4], Malihe Safari[5]

[1]Department of Oral Medicine, School of Dentistry, Arak University of Medical Sciences, Arak, Iran.
[2]Department of Pharmacology, School of Medicine, Arak University of Medical Sciences, Arak, Iran.
[3]Department of Medical Mycology and Parasitology, School of Medicine, Arak University of Medical Sciences, Arak, Iran.
[4]Dentist, Private Practice, Arak, Iran.
[5]Department of Biostatistics, School of Medicine, Arak University of Medical Sciences, Arak, Iran.

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***Corresponding author**
Department of Oral Medicine, School of Dentistry, Arak University of Medical Sciences, Arak, Iran.
Tel: +989120940783

E-mail:
momeni6384@gmail.com

Abstract

Aim: This study evaluate the antifungal effects of duloxetine as monotherapy and in combination with fluconazole on fluconazole-resistant *C. albicans*.

Methods: In this *in vitro* study, a suspension of fluconazole-resistant *C. albicans* clinical isolates from oral candidiasis was prepared using the CLSI M37-A3 method. Fluconazole (3 mg/mL) and duloxetine (160 µg/mL) stock solutions were serially diluted 9 times (0.625-160 µg/mL for duloxetine and 0.5-128 µg/mL for fluconazole). Their antifungal activity against *C. albicans* were evaluated by the microdilution method through broth media. The minimum inhibitory concentration of duloxetine, fluconazole, and their combination was also determined.

Results: Fluconazole-resistant *C. albicans* was only sensitive to 32, 64, and 128 µg/mL fluconazole and 160, 80, and 40 µg/mL duloxetine as monotherapy. It was also sensitive to 80 and 160 µg/mL duloxetine combined with all 9 dilutions of fluconazole, but 40 µg/mL duloxetine was only effective in combination with 16 to 128 µg/mL fluconazole. Fluconazole-resistant *C. albicans* was sensitive to 10 and 20 µg/mL duloxetine combined with 32, 64, and 128 µg/mL fluconazole. It was also sensitive to 0.625, 1.25, 2.5, and 5 µg/mL duloxetine combined with 64 and 128 µg/mL fluconazole. The MIC of duloxetine was 40 µg/mL as monotherapy and 16 µg/mL combined with fluconazole.

Conclusion: Duloxetine had antifungal effects and the combination of duloxetine with fluconazole had synergistic effects on inhibited fluconazole -resistant *C. albicans*.

Keywords: *Candida Albicans*, Fluconazole, Duloxetine.

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Introduction

Fungal infections have attracted the attention of researchers due to an increase in their related morbidity and mortality rates. Although species of this genus may live as members of the microbiota in healthy individuals, they may cause life-threatening infections in hospitalized and immunosuppressed patients (1). *Candida* spp. are among the most common clinically important opportunistic pathogenic yeasts (1). *Candida* spp. is responsible for a high rate of morbidity and mortality worldwide, particularly among the immunocompromised patients. They can cause vaginitis, oral candidiasis, cutaneous candidiasis, candidemia, and systemic infection (2-6). The increased resistance rates to antifungals, the high biofilm production capacities, and the fact that certain *Candida* species are inherently resistant to some antifungals suggest that new antifungal molecules are needed for therapy. Because of the eukaryotic cell structures of fungal pathogens, antifungals should have selective mechanisms that target specific structures in microorganisms different from human cells. This situation makes it difficult to develop new antifungal agents. Consequently, it is becoming more and more beneficial to investigate the antifungal and antibiofilm activities of various molecules used for diverse therapeutic purposes (1). Oral candidiasis is among the most common opportunistic infections caused by *C. albicans* and some other *Candida* species. Candidiasis often manifests as a mild disease of oral mucosa; however, in certain cases, it may resist treatment or recur. *Candida* is a member of the oral microbiota in 75% of individuals with no known underlying condition. *Candida* colonization of the oral cavity often occurs at birth, and is the highest in infants, children, and the elderly. An imbalance between the host and *Candida* due to unfavorable changes in oral microbiota (dysbiosis) or injury to anatomical or physicochemical barriers may enhance the development of candidiasis, which is alarming in the elderly, patients with underlying conditions, and particularly immunocompromised patients. Although over 150 *Candida* species have been identified so far, *C. albicans* is responsible for 95% of candidiasis cases. Other species such as *C. glabrata* and *C. krusei*, among others, can cause disseminated infections that compromise the management of candidiasis (7). *C. albicans* is isolated from the oral cavity of 60% of patients over the age of 60 years. It can cause denture stomatitis, angular cheilitis, and median rhomboid glossitis. Secondary oral candidiasis may also occur (8). Candidiasis can cause oral burning sensation, dysgeusia, dysphagia, loss of appetite, and weight loss, leading to malnutrition and decreasing the quality of life. Oral candidiasis can be pseudomembranous, erythematous, and chronic hyperplastic candidiasis. Pseudomembranous candidiasis is common in chronically ill patients and infants. It is presented as white, soft, slightly elevated plaques most commonly on the tongue and buccal mucosa. Plaques resemble curd and consist of tangled masses of fungal hyphae with intermingled desquamated epithelium, necrotic debris, keratin, leukocyte, fibrin, and bacteria. This white plaque, when wiped away, leaves an erythematous area. Erythematous candidiasis is also known as antibiotic sore mouth. It occurs as a sequel to the use of broad-spectrum antibiotics or corticosteroids. The lesions present as consistently painful erythematous areas

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along with central papillary atrophy of the tongue. It is also known as a kissing lesion when the palate is involved and exhibits erythema due to contact with the tongue. Chronic hyperplastic candidiasis, also known as candidal leukoplakia, presents with firm white persistent plaques on lips, tongue, and buccal mucosa. These plaques may be homogenous or nodular and persist for years. It has premalignant potential (8).

The contribution of basic science to the COVID-19 pandemic could not be underrated. Basic science research has rapidly developed diagnostic tests that identify infected people; a vaccine to prevent virus spread has been realized, and severe cases of the disease have received treatments from such research (7). For example, the mRNA technology used in the Pfizer-BioNTech, Noora, and Moderna vaccines was based on decades of basic science research on the biology of viruses and the human immune system (8).

Treatment of candidiasis is often comprised of proper oral hygiene, application of topical and local antifungal agents, and systemic medications (9). Two main classes of antifungal agents including azoles and polyenes are used for the treatment of candidiasis (10). Azoles are the first line of treatment for *Candida* infections (11). The most commonly prescribed azoles include fluconazole, itraconazole, and voriconazole. Since azoles (and particularly fluconazole) are commonly used in clinical practice due to their high efficacy and low cytotoxicity, development of resistance against them has become a major concern for clinicians (12). Polyenes are the first-line treatment for oral candidiasis (13). Polyenes achieve fungicidal activity by binding ergosterol in the cell membrane, resulting in increased permeability and the leakage of intracellular components, which subsequently leads to cell death (2). Amphotericin and nystatin are the most frequently prescribed polyenes. Amphotericin should only be administered in a hospital setting, and can cause thrombophlebitis, loss of appetite, nausea, vomiting, fever, headache, weight loss, insomnia, hypokalemia, renal toxicity, hypotension, and arrhythmia when administered intravenously. Amphotericin B is the most clinically relevant polyene for invasive fungal infections, and maintains a broad spectrum of fungicidal activity, covering yeasts, molds, and dimorphic fungi. Its use in practice is limited by a lack of an oral formulation, infusion reactions, and significant dose-limiting toxicities such as nephrotoxicity. The development of several lipid-based formulations has improved patient tolerability but has not completely eliminated toxicities. Despite these drawbacks, amphotericin B sees consistent clinical use as empiric coverage of invasive fungal infections until a more tolerable therapy or formulation can be identified (2). Nystatin is an oral topical medication used for the treatment of oral candidiasis. It is supplied in the form of an oral suspension, which should be rinsed regularly 3-4 times/day. It has a bitter unbearable taste, and may cause nausea and vomiting. Thus, its consumption requires high patient cooperation (13).

The toxicity of the currently available antifungal medications and the increasing prevalence of *Candida* infections by pathogens that are mostly resistant to antifungal agents, as well as the emergence of drug-resistant non-*albicans Candida* spp. highlight the need for other more efficient antifungal agents with alternative mechanisms of

action and lower cytotoxicity (2, 14-16). However, the allocated financial resources for research in this field are limited, and the process of gaining approval for clinical use is long and costly (3, 17, 18). Thus, a promising strategy would be to use a combination of the currently available medications (3, 19). The antifungal activity of selective serotonin reuptake inhibitors (SSRIs) and tricyclic antidepressants against several fungal species particularly *C. albicans* has been previously confirmed (1, 11, 12, 20-27). Also, it has been shown that tramadol is effective against *C. albicans* (28). A previous study showed that duloxetine hydrochloride in combination with fluconazole decreased the proliferation and capsule size of *Cryptococcus neoformans* (29). Duloxetine is a SNRI and a selective norepinephrine reuptake inhibitor that inhibits the reuptake of serotonin. Duloxetine is better tolerated than other SNRIs, and does not have any cardiovascular toxicity (30). It is mainly used for major depressive disorder, generalized anxiety disorder, fibromyalgia, diabetic peripheral neuropathy, and chronic musculoskeletal pain. The off-label indications of duloxetine include peripheral neuropathy due to chemotherapy and stress urinary incontinence (31). The gradually increasing fungal resistance to azoles calls for novel therapeutic strategies. To save time and cost, off-label application of the currently available medications such as antidepressants for this purpose may be a great option, given that their optimal efficacy and safety are confirmed. Considering the gap of information regarding the effects of duloxetine on fluconazole-resistant *C. albicans*, this study aimed to assess the antifungal effects of duloxetine as monotherapy and in combination with fluconazole on fluconazole-resistant *C. albicans*.

The novelty of this study is the deletion of resistant *Candida* infections by other unusual drugs that have fewer side effects and less interaction with other drugs by local use of duloxetine.

Methods

This *in vitro*, experimental study was conducted on standard-strain fluconazole-resistant *C. albicans* clinical isolates. The study protocol was approved by the ethics committee of the Arak University of Medical Sciences (IR.ARAKMU.REC.1401.332).

The sample size was calculated to be 10 in each group according to a study by Menezes et al, (29) assuming alpha=0.05, d=0.6, and a study power of 80%.

Preparation of fungal suspension

Standard-strain fluconazole-resistant *C. albicans* clinical isolates were cultured on Sabouraud dextrose agar containing chloramphenicol, and incubated at 37°C for 24 hours. Next, grown colonies were mixed with 1000 µL of sterile phosphate buffered saline in a 1.5 mL microtube. Accordingly, a fungal suspension with 1 x 106 cells/mL was prepared by using a Neubauer chamber.

Preparation of stock solutions

A total of 160 mg of duloxetine powder (Sobhan Pharmaceuticals, Iran) was dissolved in 1 L of dimethyl sulfoxide and stored at room temperature for 30 minutes to prepare sterile duloxetine stock solution (160 µg/mL) according to Menezes et al (29). Also, 128 mg of fluconazole powder (Amin Pharmaceuticals, Iran) was dissolved in 1 L of dimethyl sulfoxide and 1 L of RMPI separately, and stored at room temperature for 30 minutes to prepare

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a sterile stock solution (128 µg/mL) according to Caldara and Marmiroli (27). Accordingly, 1 sterile vial of duloxetine stock solution and 2 sterile vials of fluconazole stock solution were prepared and stored at -70°C for later use.

Serial Serial dilutions of duloxetine

Nine serial dilutions of duloxetine were prepared by the serial dilution technique. A sampler was used to transfer 10 mL of the duloxetine stock solution (160 µg/mL) to the first tube. Next, 5 mL of the contents of the first tube was transferred to the second tube, and the volume was reached 10 mL by adding dimethyl sulfoxide. The same was repeated until the 9th tube. Finally, 5 mL of the contents of the 9th tube was discarded. Accordingly, 160, 80, 40, 20, 10, 5, 2.5, 1.25 and 0.625 µg/mL concentrations of duloxetine were prepared.

Serial dilutions of fluconazole

The process was the same as that reported for duloxetine with the difference that initially, 10 mL of 128 µg/mL concentration of fluconazole was added to the first tube, and 128, 64, 32, 16, 8, 4, 2, 1 and 0.5 µg/mL concentrations of fluconazole were prepared as such.

Determination of minimum inhibitory concentration (MIC) of medications by the microbroth dilution technique

According to the CLSI M37-A3 method M27-A protocol, 96-well microplates were used for this purpose. Initially, 100 µL of Sabouraud dextrose broth was added to each well. Next, 100 µL of dimethyl sulfoxide was added followed by 100 µL of fluconazole-resistant *C. albicans* suspension with a density of 1 x 106 cells/mL. The test was repeated in triplicate. The 96-well plate was then incubated in a shaking incubator operating at 150 rpm at 37°C for 24 hours. After the incubation time, the growth of *C. albicans* was determined according to the turbidity of the wells as measured by an ELISA Reader (BioTek). Next, the effects of different concentrations of duloxetine in the range of 0.625-160 µg/mL were evaluated. Initially, 100 µL of Sabouraud dextrose broth was added to each well. Next, 100 µL of different concentrations of duloxetine was added to each well such that the final concentration of duloxetine in wells ranged from 0.625 to 160 µg/mL. Subsequently, 100 µL of fluconazole-resistant *C. albicans* suspension was added to each well at a density of 1 x 106 cells/mL. The last two wells in each row were considered as the negative (no *Candida* suspension) and positive (no stock solution) controls. In other words, the positive control well contained fluconazole-resistant *C. albicans* suspension and culture medium, and the negative control well contained duloxetine stock solution and culture medium. In the positive control well, turbidity was seen while the negative control well was completely clear. The test was repeated in triplicate. The microplate was placed in a shaking incubator operating at 150 rpm at 37°C for 24 hours. Next, the turbidity of the wells was assessed by an ELISA Reader, and the lowest concentration of medication preventing *Candida* growth was recorded as the MIC of duloxetine.

The same process was performed to assess the effects of different concentrations of fluconazole on *C. albicans*, with the difference that the final concentration of fluconazole in the wells ranged from

0.5 to 128 µg/mL. The microplate was placed in a shaking incubator operating at 150 rpm at 37°C for 24 hours. Next, the turbidity of the wells was assessed by an ELISA Reader, and the lowest concentration of medication preventing *Candida* growth was recorded as the MIC of fluconazole.

The same process was performed for assessment of the combined effects of duloxetine and fluconazole, such that the final concentration of duloxetine in wells ranged from 0.625 to 160 and the final concentration of fluconazole ranged from 0.5 to 128 µg/mL in each well. The negative control well contained the stock solutions of duloxetine and fluconazole. The rest of the procedure was the same as that explained earlier.

Statistical analysis

Data were analyzed by SPSS version 24 (SPSS Inc., IL, USA) using the logistic and probit regression models at 0.05 level of statistical significance.

Results

Susceptibility to duloxetine

The results showed that fluconazole-resistant *C. albicans* was sensitive to only the first three concentrations of duloxetine (160, 80, and 40 µg/mL), and was resistant to other concentrations of duloxetine.

Susceptibility to fluconazole

The results showed that fluconazole-resistant *C. albicans* was sensitive to only the first three concentrations of fluconazole (128, 64, and 32 µg/mL), and was resistant to other concentrations of fluconazole.

Susceptibility to combinations of duloxetine and fluconazole

Fluconazole-resistant *C. albicans* was sensitive to combinations of 160 and 80 µg/mL duloxetine with all 9 concentrations of fluconazole. Also, 40 µg/mL of duloxetine in combination with 128, 64, 32, and 16 µg/mL fluconazole inhibited fluconazole-resistant *C. albicans*. However, fluconazole-resistant *C. albicans* was resistant to combinations of 40 µg/mL concentration of duloxetine with lower concentrations of fluconazole. Fluconazole-resistant *C. albicans* was sensitive to 10 and 20 µg/mL concentrations of duloxetine combined with the first three concentrations of fluconazole (128, 64, 32 µg/mL), and was resistant to combinations of 10 and 20 µg/mL duloxetine with other concentrations of fluconazole.

Fluconazole-resistant *C. albicans* was sensitive to combinations of 5, 2.5, 1.25, and 0.625 µg/mL concentrations of duloxetine with the first two concentrations of fluconazole (128 and 64 µg/mL), and resistant to other concentrations.

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Table 1. Sensitivity of fluconazole-resistant *C. albicans* to duloxetine, fluconazole, and different combinations of them

Duloxetine	160µg/ml	80µg/ml	40µg/ml	20µg/ml	10µg/ml	5µg/ml	2.5µg/ml	1.25µg/ml	0.625µg/ml
Different concentrations of duloxetine	Sensitive	Sensitive	Sensitive	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
Fluconazole	128µg/ml	64µg/ml	32µg/ml	16µg/ml	8µg/ml	4µg/ml	2µg/ml	1µg/ml	0.5µg/ml
Different concentrations of fluconazole	Sensitive	Sensitive	Sensitive	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
Combination of duloxetine and fluconazole	128 µg/ml fluconazole	64 µg/ml fluconazole	32 µg/ml fluconazole	16 µg/ml fluconazole	8 µg/ml fluconazole	4 µg/ml fluconazole	2 µg/ml fluconazole	1 µg/ml fluconazole	0.5 µg/ml fluconazole
160 µg/ml duloxetine	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
80 µg/ml duloxetine	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
40 µg/ml duloxetine	Sensitive	Sensitive	Sensitive	Sensitive	Resistant	Resistant	Resistant	Resistant	Resistant
20 µg/ml duloxetine	Sensitive	Sensitive	Sensitive	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
10 µg/ml duloxetine	Sensitive	Sensitive	Sensitive	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
5 µg/ml duloxetine	Sensitive	Sensitive	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
2.5 µg/ml duloxetine	Sensitive	Sensitive	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
1.25 µg/ml duloxetine	Sensitive	Sensitive	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
0.625 µg/ml duloxetine	Sensitive	Sensitive	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant

MIC of duloxetine, fluconazole, and their combinations

The MIC of duloxetine alone was found to be 40 µg/mL, the MIC of fluconazole alone was found to be 32 µg/mL, and the MIC of duloxetine in combination with fluconazole was found to be 16 µg/mL.

Results of logistic and probit regression models

As shown in **Table 2,** the logistic regression model showed that by each one-unit increase in log dosage of fluconazole (f), the odds of sensitivity were 2.30 times the resistance of the microorganism (P<0.001). Also, by each one-unit increase in log dosage of duloxetine, the odds of sensitivity were 2.21 times of the resistance of the microorganism (P<0.001).

Table 2. Results of logistic regression model

Parameter	**Medication**	**P-value**	**Odds ratio**	**95% CI**	
				Lower bound	**Upper bound**
Log dosage	Fluconazole	<0.001	2.30	2.05	2.58
	Duloxetine	<0.001	2.21	1.97	2.46

Also considering that in the logit model, a negative coefficient means that an increase in the predictor leads to a decrease in the predicted probability. The results of probit regression model showed that an increase in dosage of fluconazole, decrease the predicted probability of sensitivity of the microorganism (P<0.001). Also an increase in dosage of duloxetine, decrease the predicted probability of sensitivity of the microorganism (P<0.001) (Table 3).

Table 3. Results of probit regression model

predictor	coefficient	p-value	95% CI	
			Lower bound	Upper bound
Fluconazole	-0.16	<0.001	-0.20	-0.11
Duloxetine	-0.11	<0.001	-0.15	-0.08

Table 4 shows the sensitivity probability for different concentrations of doses of duloxetine and fluconazole based on the probit regression model. For example, based on the results of this model, to reach 50% sensitivity of the microorganism, the dose of duloxetine was equal to 29.46mg and the dose of fluconazole was equal to 22.53 mg.

Table 4. Results of probit regression model for sensitivity probability for different concentrations of dosage

Probability of sensitivity	Dosage of Duloxetine	Probability of sensitivity	Dosage of Fluconazole
0.10	72.17	0.10	53.69
0.20	57.51	0.20	42.99
0.25	51.94	0.25	38.93
0.30	46.94	0.30	35.28
0.40	37.90	0.40	28.69
0.50	29.46	0.50	22.53
0.60	21.02	0.60	16.37
0.70	11.98	0.70	9.77
0.75	6.98	0.75	6.12
0.80	1.41	0.80	2.06

Discussion

This study assessed the antifungal effects of duloxetine as monotherapy and in combination with fluconazole on fluconazole-resistant *C. albicans*. The results showed that fluconazole-resistant *C. albicans* was only sensitive to 32, 64, and 128 µg/mL concentrations of fluconazole and 160, 80, and 40 µg/mL concentrations of duloxetine as monotherapy. It was also sensitive to 80 and 160 µg/mL concentrations of duloxetine combined with all 9 dilutions of fluconazole, but 40 µg/mL duloxetine was only effective in combination with 16 to 128 µg/mL concentrations of fluconazole. Fluconazole-resistant *C. albicans* was sensitive to 10 and 20 µg/mL concentrations of duloxetine combined with 32, 64, and 128 µg/mL concentrations of fluconazole. It was also sensitive to 0.625, 1.25, 2.5, and 5 µg/mL concentrations of duloxetine combined with 64 and 128 µg/mL concentrations of fluconazole. The MIC of duloxetine was 40 µg/mL as monotherapy and 16 µg/mL when combined with fluconazole.

The mechanism of action of fluconazole is through the inhibition of α-14 lanosterol demethylase, which is an enzyme responsible for the biosynthesis of ergosterol. It has been demonstrated that sertraline plays a role in the organization of intracellular membranes, translation, and vesicular transport. We believe that duloxetine acts similarly to sertraline because they are both antidepressants with the same mechanism of action. One major challenge in the treatment of candidiasis with fluconazole is a development of fungal resistance and high toxicity of this medication. Thus, a combination of duloxetine and fluconazole may be suitable as an alternative since a lower concentration of both medications would be required, and resultantly, drug cytotoxicity and risk of emergence of resistant species would decrease. Another advantage is the higher efficacy of a combination of different mechanisms of action against the yeast, yielding more favorable results. In the mechanism of resistance, expression of MDR1, as an efflux pump, results in efflux of fluconazole from the cells. It appears that duloxetine inhibits the MDR1 pump and decreases the efflux of fluconazole from the cells. It also inhibits the toxic ergosterol synthesis pathway in fungal resistance mechanism,

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and improves the efficacy of fluconazole as such. Moreover, it is believed that duloxetine can inhibit vesicular transport; although further investigations are warranted in this respect (29).

Menezes et al. (29) demonstrated that a synergistic combination of duloxetine hydrochloride and fluconazole decreased fungal growth and the size of the capsule of *Cryptococcus neoformans*. The MIC and minimum fungicidal concentration of duloxetine were both 18.5 µg/mL. Combination with fluconazole decreased the MIC by 16 times for duloxetine and by 4 times for fluconazole. The capsule size also decreased by 67% in treatment with duloxetine and 16% in treatment with duloxetine plus fluconazole. Their results were in agreement with the present findings, highlighting the significant antifungal effects of duloxetine, compared with fluconazole, and their synergistic effect (29).

Tekintaş et al. (1) demonstrated that SSRIs (sertraline, paroxetine, and fluoxetine) had antifungal and antibiofilm effects both as monotherapy and in combination with fluconazole. They evaluated 20 *Candida* spp. Sertraline showed the highest antifungal activity while the antibiofilm effect of fluoxetine was higher than others. Fluoxetine and paroxetine showed synergistic effects with fluconazole against 13 and 19 species, respectively. Pereira et al. (32) demonstrated *in vitro* synergistic effects of fluoxetine and paroxetine in combination with amphotericin B against *Cryptococcus neoformans*. Gowri et al. (33) indicated that sertraline inhibited the growth and proliferation of *C. auris* and its biofilm *in vitro*. They also revealed that the binding of sertraline to alpha-14 demethylase sterol was involved in the inhibition of the biosynthesis of ergosterol. Silva et al. (20) reported a MIC of 20-160 µg/mL for fluoxetine, 10-20 µg/mL for sertraline, and 10-100.8 µg/mL for paroxetine against fluconazole-resistant *Candida* species. Flow-cytometric assessments revealed that SSRIs damage the plasma membrane and mitochondrial membrane of yeasts and activate the apoptosis signaling pathways, resulting in dose-dependent loss of cell viability. Also, fluoxetine decreased the mature biofilm of all tested species (20). Gu et al. (12) evaluated the synergistic effects of fluoxetine in combination with azoles against *C. albicans* both *in vitro* and *in vivo*, and confirmed their synergistic effects against fluconazole-resistant *Candida* spp. However, no such effect was recorded for non-*albicans Candida* spp. Fluoxetine and fluconazole also showed synergistic effects against 4, 8, and 12-hour biofilms. It was demonstrated that fluconazole combined with fluoxetine down-regulated SAP1 to SAP4 and decreased resistant *C. albicans* species by the activity of extracellular phospholipases(12). de J Treviño-Rangel et al. (34) evaluated the antifungal effects of sertraline against *Cryptococcus spp.*, and evaluated its *in vivo* activity in a rat model of cryptococcal meningoencephalitis. They demonstrated the favorable antifungal effects of sertraline on *Cryptococcus neoformans*, and showed that its 15 mg/kg dosage decreased the fungal count in the brain and spleen with an efficacy comparable to that of fluconazole (34).

Evaluation of *C. albicans* alone among different *Candida* spp. was a limitation of this study. Also, this study had an *in vitro* design, which limits the generalizability of the findings to the clinical setting. Thus weak points of this study were the evaluation of

C. albicans alone among different *Candida* spp and not evaluation *in vivo* study in oral cavity but the strong point of this study was find of a new drug for resistant candidiasis. Future studies are required on different *Candida* spp. Also, since the majority of available studies have evaluated the antifungal effects of SSRIs, future studies should address the antifungal effects of selective norepinephrine reuptake inhibitors. *In vivo* studies are also required to obtain more generalizable results.

Conclusion

Monotherapy with 40, 80, and 160 µg/mL concentrations of duloxetine and a combination of its 80 and 160 µg/mL concentrations with all 9 concentrations of fluconazole, and also monotherapy with 32, 64, and 128 µg/mL concentrations of fluconazole and combination of its 64 and 128 µg/mL concentrations with all 9 concentrations of duloxetine inhibited fluconazole-resistant *C. albicans*. Thus duloxetine had antifungal effects and a combination of duloxetine with fluconazole had synergistic effects on inhibited fluconazole-resistant *C. albicans*.

Declarations

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Conflict of Interest

The authors declare that they have no conflicts of interest.

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Ethics approval and consent to participate The protocol was approved by the Arak University of Medical Sciences Ethics Committee (IR.ARAKMU.REC.1401.332).

Authors' contributions

EM, AT, and MD contributed to the study conception and design. Material preparation and data collection were performed by AT, EM, and AA. Analyses were performed by MS. The first draft of the manuscript was written by EM and AT. All authors read and approved the final manuscript.

Consent for publication

Not applicable.

Informed consent

Not applicable.

Availability of data and materials

The datasets used during the current study are available from the corresponding author upon reasonable request.

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