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Improved Yeast Delivery of Fluconazole with a Nanostructured Lipid Carrier System

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Abbreviations[1]

[1]**Abbreviations: MIC:** Minimum Inhibitory Concentration; **FLZ:** Fluconazole; **FLZ-NLC:** Fluconazole loaded Nanostructured Lipid Carrier; **NLC:** Nanostructured Lipid Carrier.

Abstract:

Despite the growing trends in the number of patients at risk for invasive fungal infections, management with current antifungal agents results in complications due to changes in the epidemiology and drug susceptibility of invasive fungal infections. In the present research fluconazole-loaded nanostructured lipid carriers were prepared using probe ultrasonication techniques and investigated the efficacy of the optimal formulation on a large number of *Candida* species. The morphology of the obtained nanostructured lipid carriers was characterized by transmission-electron microscopy. The minimum inhibitory concentrations (MIC) for the new formulations against strains of *Candida* were investigated using the Clinical and Laboratory Standards Institute document M27-A3 and M27-S4 as a guideline. The fluconazole-loaded nanostructured lipid carriers presented a spherical shape with a mean diameter, zeta potential and entrapment efficiency of 126.4 ± 15.2 nm, -35.1 ± 3.0 mV, and 93.6 ± 3.5%, respectively. The drug release from fluconazole-loaded nanostructured lipid carriers exhibited burst-release behavior at the initial stage followed by sustained release over 24 hours. Using a new formulation of fluconazole led to a significant decrease in MICs for all *Candida* groups ($P < 0.05$). Furthermore, *C. albicans* isolates showed more susceptibility to fluconazole-loaded nanostructured lipid carriers than *C. glabrata* and *C. parapsilosis* ($P < 0.05$). The MIC_{50} drug concentration was obtained as 0.0625, 0.031 and 0.25 μg/ml for fluconazole-resistant strains of *C. albicans*, *C. glabrata*, and *C. parapsilosis*, respectively. In conclusion, a novel delivery system which can be used as part of a strategy to improve the antifungal activity of fluconazole against various Candida strains with different susceptibilities to conventional formulations of fluconazole was evaluated.

Keywords: Fluconazole, Drug delivery, Nanostructured Lipid Carrier, *Candida*

1. Introduction:

A variety of novel drug carrier systems have recently been proposed to improve the bioavailability and release of drugs [1]. These systems are aimed at maintaining local effects and enhancing drug accumulation in various strata of skin through liposomes or niosomes [2], gel formulation[3], lecithin-based organogel [4], hydrogel [5] or polymeric mucoadhesive films, [6] poly-lactic co-glycolic acid (PLGA) microspheres [7], solid lipid nanoparticles (SLNs) [8, 9], and nanostructure lipid carriers (NLCs) [10]. In particular, NLCs have emerged as a promising drug delivery system for pharmaceutical and cosmetic molecules, especially for the delivery of lipophilic compounds.[11-13] Briefly, NLCs are colloidal nanocarriers in the submicron range (40 to 1000 nm) composed of a solid lipid matrix and a liquid lipid [13]. They have several desirable advantageous such as low toxicity of constituents, and the ability to protect the incorporated drug from degradation by immobilization in the solid/liquid particle matrix [14]. Moreover, NLCs have overcome problems associated with SLNs, including limited drug loading, risk of gelation, and drug leakage during storage caused by lipid polymorphism [14].

Fluconazole (FLZ), a first-generation triazole, is a broad-spectrum anti-fungal agent that inhibits cytochrome P450-dependent 14α-lanosterol demethylation, a vital step in cell membrane ergosterol synthesis [15]. Fluconazole is active against all *Candida* species except for *C. glabrata*, which has acquired resistance to fluconazole, and *C. krusie*, which is resistant to the drug intrinsically [16, 17]. Despite its advantageous pharmacological activity, FLZ can cause several clinically significant side effects, including headache, hives, itching or skin rash, abdominal pain, and hematemesis [3]. While the prevalence and severity of side effects may be decreased by lowering the dose of FLZ, clinical efficacy may be reduced and resistance increased through this approach. This problem is important because FLZ is currently used for

both prophylaxis and the treatment of broad spectrum infections of candidiasis, yet, the emergence of drug-resistant isolates continues to increase dramatically [18-20]. In response to this challenge, the use of new drug formulations and drug delivery systems to reduce resistance while maintaining or increasing clinical efficacy are urgently needed [21] [22]. While there is limited evidence regarding the effectiveness of FLZ loaded NLCs (FLZ-NLC) [1] on *Candida albicans*, the activity of FLZ-NLCs against a broad range of *Candida* species, including *C. glabrata* and *C. krusie*, has not yet been studied. To this end, the purpose of the present study was to estimate the clinical efficacy of FLZ-NLCs on FLZ-resistant strains of certain Candida species.

2. Materials and Methods

2.1 Materials

Fluconazole (FLZ, Pharmaceutica grade) was obtained from Arasto Pharmaceuticals Chemicals Inc. (Tehran-Iran). Compritol® 888 ATO (CO), Lipocire and Precirol® ATO 5 were supplied from Gattefossé (Saint-Priest, Cedex, France). Sabouraud dextrose agar (SDA), RPMI medium, stearic acid (SA), Oleic acid, Tween 80 (Tn80), Span 60 (Sn60) and Span 80 (Sn80) were purchased from Merck Co. (Germany). HPLC grade acetonitrile and methanol were supplied by the Merck (Germany). Morpholinepropanesulfonic acid (MOPS) was purchased from Sigma Chemical Co., St. Louis, MO (USA). Deionized water was purified using a Milli-Q system (Millipore, Direct-Q). All other reagents and solvents were either of analytical or high-performance liquid chromatography (HPLC) grades.

2.2. Screening of lipids

In order to determine the maximum amount of drug a lipid can hold, it is necessary to estimate the solubility of the given drug in the lipid. To accomplish this goal, we evaluated the solubility of FLZ 25 %w/w in melted Compritol® 888 ATO, Lipocire, Precirol® ATO 5 and stearic acid (SA) (Merck, Germany). The solubility of FLZ was also assessed in melted solid lipid combined with oleic acid as a liquid lipid, in ratios of 90:10, 80:20 and 70:30. The lipid mixtures were stirred at 200 revolutions per minute for 10 minutes at 85°C, using a hot plate magnetic stirrer (Unimax 1010, Heidolph, Germany). When the drug was dissolved in lipids with different concentrations, they were examined for the presence of the drug crystals in the lipid matrix to explore which lipids or lipid combinations were able to dissolve the drug completely.

2.3. Preparation of formulation

FLZ-NLCs were prepared and optimised via a process utilising an ultrasonic probe. The method has been adopted from the previously reported studies in the literature [10]. To prepare NLCs, a carrier lipid in its solid form (stearic acid/ Compritol® 888 ATO) was melted at 85 °C in combination with liquid lipid (oleic acid) and a lipophilic surfactant (Span 80) based on the proportion in Table 2. The molten lipid phase was dispersed in a 1/3 of the aqueous solution of hydrophilic surfactant prepared by weighing out 0.84 % w/w Tween 80 at the same temperature and sonicated by using a probe sonicator (Bandelin sonopuls, Berlin, Germany) for 5 min (Model HD 3200, Prob TT25, 50% power and 14.28 KJ, continuous) to form a pre-emulsion. At the end of the sonication, the mixture was dispersed into the remaining 2/3 of the hydrophilic surfactant solution maintained in an ice bath. The final mixture was sonicated again for 10 min (50 % power and 43.21 KJ) whilst still immersed in the ice-bath (Table 2).

FLZ-loaded NLCs were prepared using the probe ultrasonication method, which has been used previously in the production of lipid nanoparticles [10]. Briefly, solid lipid either alone or in combination with oleic acid and Span 80 (Sn80) (Merck, Germany) were melted at 85°C. FLZ was then added to the lipid and the mixture was stirred until the drug was completely dissolved. An aqueous phase composed of deionized water and Tween 80 (Tn80) at 85°C was added to the lipid phase using Ultra-Turrax® (IKA, Heidelberg, Germany), a high-shear stress homogenizer, for 5 minutes at 10,000 revolutions per minute. The heated mixture was then sonicated (Bandelin Sonopuls, Berlin, Germany) for 5 minutes. At the end of the sonication, the mixture was dispersed into an ice-cold surfactant solution maintained in an ice bath. The final mixture was sonicated again for 10 minutes while immersed in the ice-bath to promote the formation of the lipid nanoparticles (Table 2).

2.4. Characterization of the nanoparticles

2.4.1. Morphology

In order to determine the shape of FLZ-NLCs, transmission electron microscopy **(accelerating voltage 100kV; TEM**, CM 30, Phillips, Netherlands) was used. First, the NLC samples were diluted two times with distilled water. One drop of the diluted sample was placed on a 200-mesh carbon-coated copper grid, stained with 2 % phosphotungstic acid solution and dried at room temperature. Representative images of the sample were reported.

2.4.2. Particle size and zeta potential

Photon correlation spectroscopy (PCS) with a Malvern Zetasizer ZS (Nano ZA, Malvern Instruments, UK) was used to determine the particle size, and to profile the size distribution

(polydispersity index, PDI) and zeta potential of the nanoparticles. In this method, the sample was measured at 25 °C with an angle detection of 90°. The concentration of the samples for analysis on the Zetasizer was 20-400 kilocounts per second (KCPS) and the intensity of diffraction was 100,000 counts per second.

2.4.3. High-performance liquid chromatography (HPLC) analysis of fluconazole

The HPLC assay was performed using an Agilent 1100 chromatograph, equipped with the Agilent Eclipse XDB-C18 column (5 µm, 4.6 mm × 250 mm). The mobile phase was composed of 10 mM sodium acetate buffer (adjusted to pH 5.0 with glacial acetic acid) and methanol (65:35) with a flow rate of 1 ml/minute.

2.4.4. Determination of fluconazole entrapment efficiency

Entrapment efficiency (EE%) was determined to assess the extent of FLZ incorporation in the nanoparticles by measuring the concentration of the free unloaded FLZ in the aqueous phase of the nanoparticle suspension. To determine the entrapment efficiency (EE%) of FLZ in the NLCs, the FLZ-NLCs were subjected to centrifugation for 20 minutes at 25,000 rpm (HERMLE, Z36HK, Germany) and filtered (pore size: 0.22 µm). The amount of drug in the supernatant was determined by HPLC and the experiment was conducted in triplicate.

The drug entrapment efficiency (EE%) was calculated from Equation 1, where Wi and Wf were the amount of drug added in the formulation and amount of drug in the supernatant, respectively.

$$EE\% = \frac{Wi - Wf}{Wi} \times 100 \quad \text{(Eq. 1)}$$

2.5. Drug release measurement

The release of FLZ was estimated using the dialysis tube technique. To determine the release rate of FLZ from the nanoparticles, 5 ml of the prepared dispersion was poured into a dialysis bag using a cut-off of 12,500 Daltons, with the two ends fixed by clamps. The bag was then dropped into 500 ml of simulated intestinal fluid at a pH of 6.8 and stirred with a magnetic stirrer at 60 revolutions per minute at 37.0 ± 0.1°C. Samples were withdrawn at predetermined time intervals of 30 min and 1, 2, 4, 6, 8 and 24 hours, and replaced with fresh simulated intestinal fluid maintained at the same temperature. Samples of 1.5 ml were withdrawn, centrifuged for 30 minutes at 25,000 revolutions per minute, filtered with a 0.22 µm filter, and directly injected into the HPLC system.

2.6. Antifungal susceptibility testing for fluconazole-loaded nanostructure lipid carriers

2.6.1. Isolates

To assess the effectiveness of NLCs containing FLZ, we applied FLZ-NLCs against 90 clinical *Candida* isolates as a means of protecting the drug binding sites. All strains were originally isolated from Iranian patients suffering from cutaneous and sub-cutaneous candidiasis (all *C. albicans* and *C. parapsilosis* strains were vaginal isolates; the rest of *Candida* strains were isolated from vagina, bronchoalveolar lavage, sputum, and nail) and were identified to the species level by a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis using an *Msp*1 restriction enzyme [23]. Resistant strains were proven to be resistant against FLZ (data not shown) by using standard protocol CLSI documents M27-A3 and M27-S4. Stock cultures were initially grown on yeast extract peptone dextrose agar (YEPD; 1%

184 yeast extract, 2% Bacto Peptone, 2% dextrose) at 35°C for 48 hours to obtain fresh viable yeast
185 cells. To perform antifungal susceptibility testing, the isolates were inoculated onto Sabouraud
186 dextrose agar (SDA; supplied by Merck, Germany) and incubated at 35°C for 24 hours.
187
188 2.6.2. Antifungal agents
189 Antifungal susceptibility testing (AFST) was performed with FLZ, as well as FLZ-NLCs.
190 Fluconazole (Arasto Pharmaceuticals Chemicals Inc., Iran) was dissolved in sterile water, and a
191 two-fold dilution was prepared in Roswell Park Memorial Institute (RPMI) 1640 medium (with
192 L-glutamine, without bicarbonate) (Merck, Germany) and buffered to pH 7.0 using a 0.165 M
193 solution of 3-N-morpholinepropanesulfonic acid (MOPS; Sigma Chemical Co., St. Louis, MO).
194 Two-fold dilutions of FLZ-NLC were prepared with equal concentrations of FLZ such that 16
195 µg/ml to 0.1 µg/ml. **FLZ**-SLNs were freshly synthesized and applied for AFST for one week
196 afterward.
197
198 2.6.3. Antifungal susceptibility testing
199 A minimum inhibitory concentration (MIC) against both FLZ and FLZ-NLCs was determined
200 according to recommendations confirmed in the CLSI M27-A3 and M27-S4 documents [24, 25].
201 **FLZ** and FLZ-NLCs were dispensed into 96-well microdilution trays at a final concentration of
202 0.063–64 g/ml and 0.1–8% (0.1–8 g/ml), respectively. *Candida* blastospore suspensions were
203 prepared from isolates grown for 24 hours. Yeast colonies were suspended in sterile saline
204 solution and spectrophotometrically adjusted at 530 nm to optical densities (OD) from 0.09 to
205 0.13 (absorbance at 530 nm ranging from 0.09 to 0.13 is equal to transmission of 75–77%). The
206 final size of the stock inoculums ranged from 1 × 106 to 5 × 106 CFU/ml. A working suspension

was then made using a 1:10 dilution followed by a 1:100 dilution of the stock suspension with RPMI medium, which resulted in two times test inoculums (1×10^3 to 5×10^3 CFU/ml). Microdilution plates were incubated at 35°C and examined visually after 24 and 28 hours as the concentration of drug that elicited significant inhibition (approximately 50%) of growth compared with a drug-free control. In the case of FLZ-loaded NLCs, results were examined using an inverted microscope (Motic AE31, Hong Kong, China), due to its self-turbidity. *C. parapsilosis* (ATCC 22019) was chosen as the quality control to be used with each new series of MIC plates.

2.7. Statistical analysis

All of the results were expressed as the mean ± standard deviation. The groups were compared by an analysis of variance (ANOVA) following Dunnett's test. In order to determine if FLZ-NLCs could significantly decrease the MIC values, a statistical analysis of the results was performed using the Mann-Whitney U test for studying the differences between the MICs of susceptible and resistant *Candida* isolates and the Kruskal–Wallis test for three groups of *Candida* strains (*C. albicans*, *C. parapsilosis* and *C. glabrata*). The differences were considered statistically significant at $P < 0.05$. The statistical analysis was performed using SPSS (V18).

Results and Discussion

2.8. Screening of lipids

After evaluating the solubility of FLZ in the lipids, four lipids with different physicochemical properties were selected for the formulation of lipid nanoparticles, as listed in Table 1. Since no drug crystals were observed when FLZ and Compritol® 888 ATO or stearic acid were heated together (for all solid lipid:liquid lipid ratios), Compritol® 888 ATO and stearic acid were selected for production of NLCs. In contrast, FLZ was not completely soluble in the other lipids listed in Table 1.

2.9. Characterization of SLNs

The particle sizes, zeta potential, and entrapment efficiency of the developed SLNs and NLCs are shown in Table 2. The FLZ-NLC-6 was selected as the best formulation because of its small particle size (126.4 ± 15.2 nm), relatively high zeta potential (-35.1 ± 3.0) and good EE% (93.6 ± 3.5) ($P < 0.05$). Furthermore, it was observed that the average particle size decreased with an increase in the concentration of oleic acid ($P < 0.05$) however there wasn't a significant difference between the two lipids in cases of zeta potential and PDI. These results were in agreement with previously reported studies which an increase in the liquid phase (oleic acid) in the formulations reduced the viscosity of the NLC suspensions and thus the particle size decreased [26]. Yuan et al. indicated that no obvious change in particle size was found when the oleic acid content in NLC was increased [27]. The solubility of the drug in the liquid lipid could probably be considered as another key factor for the reduction in particle size.

It has also been reported that the concentration of oleic acid had no significant effect on NLC particle size [28]. The phenomenon for zeta potential can be explained by the fact that oleic acid is negatively charged at its carboxylic groups. Thus, NLC-3 revealed the highest zeta potential values, possibly due to the accumulation of oleic acid at the surface of the nanoparticles [28]. It

is clear that the drug entrapment efficiency rose with increasing percentages of oleic acid (Table 2) ($P > 0.05$). This observation corresponds with findings that the incorporation of liquid lipids to solid lipids could leave enough space to accommodate drug molecules, thus, leading to improved drug entrapment efficiency [26]. The higher solubility of FLZ in liquid lipid could be another factor for the improvement of entrapment efficiency. Last, the TEM micrographs of the FLZ-NLC revealed that the FLZ-NLC particles are spherical in nature (the figure is not shown).

2.10. In vitro release study

The drug release profile of the FLZ-NLCs is shown in Figure 1A. In this study, the FLZ-NLCs showed burst-release behavior during the first 30 minutes (the initial stage), followed by a sustained release pattern. The burst release in the early stage could be due to the diffusion of the unencapsulated drug on the NLC surface and thereafter from the core. Hu et al. showed that the release rate at the initial stage increased with the increasing oleic acid content in nanoparticles and that this phenomenon is dependent on the homogeneity of the mixture of liquid and solid lipids during the NLC preparation [26].

2.11. Antifungal susceptibility testing

Using the CLSI document M27-A3 susceptibility testing methodology, the development of most strains of *Candida* species was clearly visible after 24 hours of incubation at 35°C. However, the results were interpreted after 48 hours of incubation at 35°C, as CLSI recommended. Out of 90

isolates, 39 isolates demonstrated high MIC values (≥64 µg/ml) against **FLZ** (Table 3 and Figure 2), among which, 14 isolates were *C. albicans* (35%), 13 were *C. glabrata* (33%), 9 were *C. parapsilosis* (23%), and 3 were *C. krusei* (7.7%). No statistically significant differences were seen among MICs for FLZ-susceptible *Candida* species. Table 3 depicts the results for the *in vitro* antifungal susceptibility profile of FLZ-NLCs against FLZ-susceptible isolates. Specifically, we found that using the new formulation of FLZ-NLCs leads to a statistically significant decrease in MIC values ($P < 0.05$). *C. albicans* isolates showed more susceptibility against FLZ-NLCs than *C. glabrata* or *C. parapsilosis* ($P < 0.05$). Furthermore, the MIC_{50} drug concentration was obtained as 0.031, 0.15, and 0.15 µg/ml for *C. albicans*, *C. parapsilosis* and *C. glabrata*, respectively. Table 4 shows details of the MIC for resistant strains of all species. **FLZ**-resistant strains behaved as susceptible ones after being treated with new formulations, such that their MIC values were in the susceptible range.

The MIC_{50} drug concentration was obtained as 0.0625 , 0.031 and 0.25 µg/ml for FLZ-resistant strains of *C. albicans*, *C. glabrata* and *C. parapsilosis*, respectively. Comparing the results for two susceptible and resistant groups, FLZ-NLCs were significantly more effective on FLZ-susceptible *C. albicans* and *C. glabrata* isolates. However, the activity of FLZ-NLCs was not statistically significantly different for *C. parapsilosis* strains ($P > 0.05$). Overall, FLZ-NLCs resulted in a significant decrease in MIC ($P < 0.05$).

To the best of our knowledge, there are few documented reports about the synthesis of FLZ-NLCs. Thus, studying their effect on a large number of *Candida* species is novel. The small size of the lipid particles in the NLCs helps ensure close contact with the target tissue and may

enhance the penetration of drugs [29] Moreover, the size of lipid matrices may also enable sustained drug release [30, 31]. Nanostructured lipid carriers have been developed to overwhelm the drawbacks associated with SLNs. In contrast with SLNs, NLCs show a higher loading capability for compounds, since blending a liquid lipid with the solid lipid results in a higher element drug stacking. Additionally, NLCs allow the release of the drug to be controlled and increase the chemical stability of the incorporated drugs. Furthermore, NLCs are protected carriers, which can be produced easily on a large scale [14, 32-34].

According to our results, the MICs of FLZ were significantly decreased when FLZ-NLCs were used; suggesting that the therapeutic dose and risk of adverse drug effects may be decreased. One possible explanation for these antifungal susceptibility results is the mechanism responsible for the drug resistance recognized in pathogenic fungi [35, 36]. The overexpression of plasma membrane transport proteins that pump azoles out of cells is a frequent mechanism of high-level azole resistance in fungi, thereby reducing the intracellular azole concentrations below the levels at which Erg11p is inhibited [37, 38]. Knowing the conformations of the efflux pumps may lead to the design of novel formulation/drug delivery systems for azole drugs in order to keep the drug away from the efflux pumps [39]. Therefore, description of the architecture of drug-binding sites in ABC transporters is essential to understand the drug-protein interactions. This knowledge will also contribute to understanding the design of specific inhibitors or suitable shields to prevent drug-protein interactions. In this study, FLZ-NLCs provided an effective nanoscaled safeguard that protects the drug from being pumped out by transporter proteins. Moreover, the hydrophobic surface of FLZ-NLCs may lead to the drug entering the yeast cells more efficiently.

Since there is an increasing trend in emerging the azole-resistant fungal isolates, it would be a great idea to assess the new delivery system for other azoles such as voriconazole.

3. **Conclusions**:

In this study, we evaluated a novel delivery system for combating several *Candida* strains that exhibit different susceptibility to a conventional formulation of FLZ. An FLZ-loaded NLC consisting of stearic acid was successfully prepared by probe ultrasonication technique. Novel drug formulations may avoid drug recognition by efflux pump proteins, keeping the drug away from transporters. This study was the first to report the effectiveness of FLZ-NLCs as alternative delivery systems for FLZ on *Candida* isolates in vitro. However, studies on the efficacy of the new formulation are recommended for future works in vivo. Moreover, the assessment of the stability of synthesized FLZ-NLCs is a challenging issue which may address this question whether this formulation is suitable through topical, oral or systemic use or not.

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Conflict of Interest:

There is no conflict of interest.

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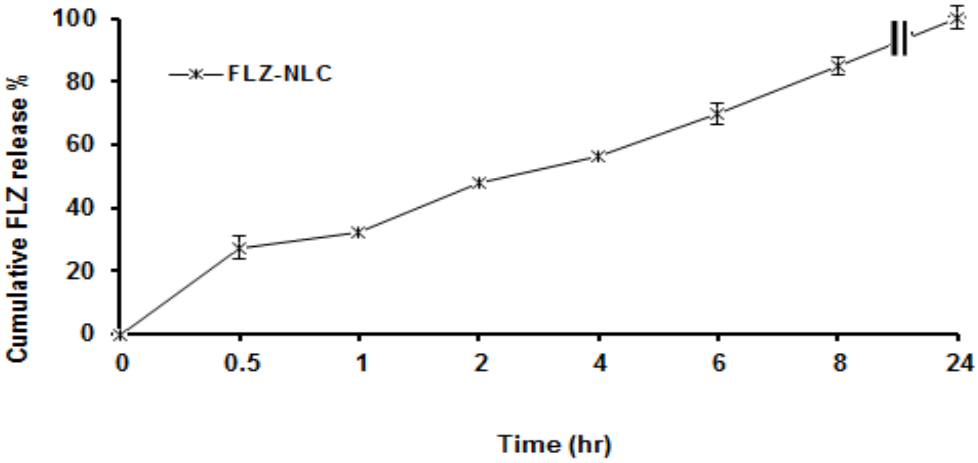

Figure 1. In vitro release of FLZ-NLCs 6 (n = 3; $P \leq 0.05$)

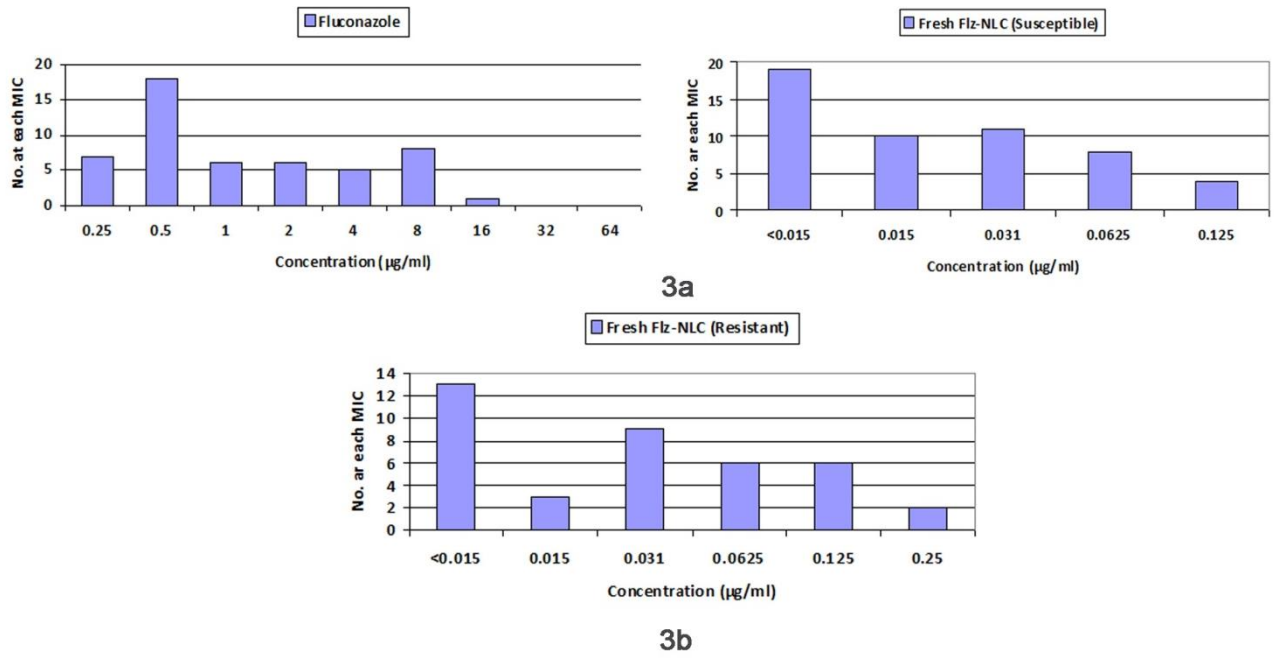

Figure 2: Comparison between the numbers of isolates at each reported MICs of susceptible strains while treating with FLZ and FLZ-NLCs (fig 3a). Fig 3b shows the number of isolates reported at each MIC in FLZ-resistant Candida species. Using FLZ-NLCs, susceptible isolates (3a) showed a significant decrease in MIC values (P value < 0.05). Moreover, resistant isolates (3b) shows increased susceptibility against FLZ-NLCs in such a way as to be placed in the susceptible range [25].

Table 1. Screening of lipids based on solubility of FLZ

Lipid 25 mg FLZ/100mg Solid: liquid lipid				
	100:00	**90:10**	**80:20**	**70:30**
Compritol® 888 ATO	+	+	+	+
Lipocire	-	-	-	+
Precirol® ATO 5	-	-	+	+
Stearic acid	+	+	+	+

Table 2. Component and physicochemical properties of investigated FLZ-loaded SLN and NLC (% w/w).

	FLZ	**CO**	**SA**	**OA**	**Sn80**	**Tn80**	**PS**	**PI**	**ZP**	**EE%**
NLC1	0.5	1.8	-	0.2	1.25	2.5	165.0±19.9	0.134±0.010	-28.1±2.6	89.1±6.3
NLC2	0.5	1.6	-	0.4	1.25	2.5	158.0±12.9	0.221±0.010	-31.6±2.6	91.7±4.2
NLC3	0.5	1.4	-	0.6	1.25	2.5	148.0±13.9	0.134±0.010	-33.6±2.6	92.3±1.3
NLC4	0.5	-	1.8	0.2	1.25	2.5	188.0±11.1	0.260±0.050	-27.3±1.8	90.0±2.6
NLC5	0.5	-	1.6	0.4	1.25	2.5	140.2±19.3	0.203±0.011	-29.1±4.3	92.0±2.6
NLC6	0.5	-	1.4	0.6	1.25	2.5	126.4±15.2	0.225±0.012	-35.1±3.0	93.6±3.5

CO: Compritol® 888 ATO; **SA:** Stearic acid; **OA:** Oleic acid; **Sn80:** Span80; **Tn80:** Tween80.

Table 3. Effect of new delivery system of FLZ on FLZ-susceptible and FLZ-resistant strains of some common *Candida* isolates

Isolates	Number (n)	Antifungal agent	MIC													MIC range	MIC50	MIC90	GM	Mode
			≥64	32	16	8	4	2	1	0.5	0.25	0.125	0.062	0.031	≤0.015					
C. albicans	20 (S)	FLZ	-	-	-	-	-	2	3	13	2	-	-	-	-	0.25-2	0.5	1.1	0.594604	0.5
		FLZ-NLC	-	-	-	-	-	-	-	-	-	2	6	9	3	0.125-0.015	0.031	0.075	0.039444	0.031
	17 (R)	FLZ	14	-	1	2	-	-	-	-	-	-	-	-	-	8-64	-	-	-	-
		FLZ-NLC	-	-	-	-	-	-	-	-	1	3	5	4	1	0.015-0.25	0.062	0.125	0.059172	0.062
C. glabrata	16 (S)	FLZ	-	-	-	6	6	1	2	-	1	-	-	-	-	0.25-8	4	8	5.656854	8
		FLZ-NLC	-	-	-	-	-	-	-	-	-	1	-	1	14	0.015-0.0125	0.015	0.015	0.015518	0.015
	13 (R)	FLZ	13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		FLZ-NLC	--	-	-	-	-	-	-	-	-	2	-	5	6	0.015-0.125	0.015	0.106	0.023019	0.015
C. parapsilosis	9 (S)	FLZ	-	-	-	-	-	3	1	2	3	-	-	-	-	0.25-2	0.5	2	1	2
		FLZ-NLC	-	-	-	-	-	-	-	-	-	-	-	-	9	-	0.015	0.015	0.015	0.015
	9 (R)	FLZ	9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		FLZ-NLC	-	-	-	-	-	-	-	-	1	-	-	-	8	0.015-0.25	0.015	0.062	0.020505	0.015
C. tropicalis (S)	2	FLZ	-	-	-	-	-	-	-	1	1	-	-	-	-	0.25-0.5	-	-	-	-
		FLZ-NLC	-	-	-	-	-	-	-	-	-	-	1	-	1	0.015-0.062	-	-	-	-
C. kefyre (S)	2	FLZ	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-
		FLZ-NLC	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-
C. krusei (R)	3	FLZ	2	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		FLZ-NLC	-	-	-	-	-	-	-	-	-	1	1	-	1	0.015-0.125	-	-	-	-

*__GM__: geometric mean MIC.

** **FLZ**: fluconazole, **FLZ_NLC**: fluconazole loaded Nanostructured Lipid Carrier