

## An In Vitro Evaluation of Anti-fungal Activity of Different Nano forms of Fluconazole Against *Candida albicans*

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The study aims to compare the antifungal effectiveness of nine different nano-particle-containing fluconazole variants with the standard form of fluconazole against *Candida albicans* in a laboratory setting. The study is an experimental laboratory-based study. The research was conducted in the department of Medical Laboratory Sciences at Gulf Medical University, Ajman. The study used standard strains of pathogenic *Candida albicans* ATCC66027 for experimentation. *Candida albicans* was exposed to varying concentrations of nine different nano-particle-containing fluconazole forms. The mixture of the organism and drug was incubated for 2 minutes, followed by transferring 50  $\mu$ L to Sabouraud Dextrose agar plates. These plates were then incubated at 37 °C for 48 hours. The primary outcome measure was the counting of colonies using a colony counter, with the number of CFUs/mL plotted against the concentration of different nano forms of fluconazole to estimate differences in effectiveness. The findings suggest that the nano form of fluconazole effectively inhibits the growth of *Candida albicans*, especially at higher concentrations, compared to the normal form of fluconazole. The study highlights that the small size of the nano agents allows for better penetration of fluconazole, enhancing its effectiveness against *Candida albicans*. The study concludes that nano-particle-containing fluconazole variants demonstrate significant variations in reducing the colony count of *Candida albicans* when compared to the standard form of fluconazole.

**Keywords:** Anti-fungal, Fluconazole, nano agents, *Candida albicans*.

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Fluconazole is a first-generation triazole antifungal. It is used to treat a wide range of fungal infections. Examples include *Candida*, blastomycosis, coccidioidomycosis, cryptococcosis, histoplasmosis, dermatophytosis, and pityriasis versicolor. It is also used to prevent candidiasis in a high-risk population, such as those

who had organ transplants, premature neonates, or have low blood neutrophil counts<sup>1</sup>.

*Candida albicans* has historically been the most common species responsible for these infections, and it continues to be a prevalent pathogen. However, there are other *Candida* species, including *Candida tropicalis*, *Candida*

glabrata, *Candida krusei*, *Candida parapsilosis*, *Candida guilliermondii*, and more, which can also cause infections. Each of these species may exhibit different characteristics, drug susceptibilities, and clinical manifestations. The rise in the prevalence of invasive systemic candidiasis, especially in immunocompromised individuals, is concerning. Factors such as the increased use of broad-spectrum antibiotics, immunosuppressive therapies, and the growing population of individuals with conditions that weaken the immune system (e.g., HIV/AIDS, cancer, organ transplantation) contribute to this trend. Additionally, *Candida* species can develop resistance to antifungal medications, which further complicates treatment.

Efforts in healthcare and research are ongoing to better understand these fungal infections and to develop improved strategies for prevention and treatment. Early diagnosis and appropriate antifungal therapy are crucial for managing candidiasis and reducing its impact on vulnerable patient populations. This may involve a combination of antifungal drugs, depending on the species of *Candida* involved and their susceptibility to different medications<sup>2</sup>.

The increasing number of reports from around the world about drug resistance among fungi and *Candida* species, combined with the production of new antifungal drugs, indicates the need for testing susceptibility to these drugs and makes researchers eager to determine susceptibility patterns for the various antifungal drugs<sup>1-3</sup>. Recently, the synthesis of nanoparticles by microorganisms has been recognized as a viable option for large manufacturing of nanoparticles<sup>4,5</sup>. These particles are utilized to transport antifungal drugs such as itraconazole<sup>6-8</sup>. Because of their tiny size, these lipid nanoparticles have more access to tissues and have greater effect<sup>9-12</sup>. These drug delivery methods provide regulated drug release, enhancing the chemical stability of the trapped pharmaceuticals. Furthermore, these systems are among the safest and most secure carriers that can be mass-produced on a huge scale<sup>13-15</sup>. The effect of nano agents is compared to the regular form of Fluconazole in this study to determine the efficacy and comparability.

Nanovesicles are widely used to deliver and/or target active ingredients to different body organs and tissues<sup>16</sup>. A wide range of developed

nanovesicles includes liposomes, niosomes, ethosomes, transfersomes, cubosomes, and micelles<sup>17-19</sup>. They have been acting as a platform for improving drug solubility, stability, release profile, and bioavailability<sup>20</sup>. These nanovesicles have been optimized to be taken by all routes of administration including oral, buccal, nasal, ocular, transdermal, as well as parenteral routes. Further modifications have been performed to develop vector-oriented nanovesicles for drugs targeting to colon, brain, liver, and tumor<sup>21</sup>.

In the current study, the antifungal activity of nano-fluconazole was compared to normal aqueous fluconazole on *Candida albicans* which is clinically important fungi.

## MATERIAL AND METHODS

This research was carried out in microbiology lab at Gulf Medical University, Ajman. It is an experimental study that aims to emphasise the effect of nano-fluconazole forms on *Candida albicans*. It is a pilot study performed using a control strains of *Candida albicans* ATCC 66027. The institutional IRB approved the study in accordance with the GMU research policies.

### Procedure of the study

The pellet of lyophilized *Candida* is reconstituted as per the instruction of the ATCC.

### Preparation of fluconazole nanovesicles

The nanovesicles were prepared in the college of Pharmacy at GMU using a modified thin film hydration technique<sup>22</sup>. Briefly, fluconazole, cetyl alcohol and Tween 80 were precisely weighed, dissolved in 10 mL mixture of methanol: chloroform, in a ratio of 2:1 v/v, and transferred into 250 mL round-bottom flask. Under vacuum, the organic solvents mixture was evaporated using the rotary evaporator (Rotavapor, Heidolph VV 2000, Burladingen, Germany) rotating at 150 rpm for 20 min at temperature 60 °C. The wall-assembled thin film has been hydrated under normal pressure using 10 mL aqueous solution of low molecular weight chitosan in 0.1 M acetic acid. Finally, the prepared nanovesicles were sonicated in ultrasonic bath (Model SH 150-41, PCI Analytics Pvt. Ltd, Mumbai, India) for 1 minute to avoid aggregation<sup>23</sup>.

### Statistical Design

Central composite design was utilized to study the effect of the formulation variables on

the characteristics of prepared nanovesicles using Design-Expert® 7 software (Version 7, Stat-Ease Inc., MN). Two independent factors were studied as follows: Tween/Cetyl ratio (X1) and chitosan percentage (X2). The traced responses were the vesicular size (PS, Y1), polydispersity index (PDI, Y2), zeta potential (ZP, Y3) and encapsulation efficiency (EE, Y4). Moreover, desirability values were estimated for selection of the optimized formulation<sup>24</sup>.

#### Characterization of the prepared fluconazole nanovesicles Analysis of vesicular size, polydispersity index and zeta potential

Dynamic light scattering adopted in the Zetasizer (Nano ZS, Malvern Instruments, Malvern, UK) were utilized for the analysis of the PS, PDI and ZP of the nanovesicular formulations. Samples taken from each formulation were diluted until being hazy before analysis.

#### Determination of the encapsulation efficiency of the prepared fluconazole nanovesicles

Fluconazole-loaded *nanovesicles* was centrifuged and separated from the un-encapsulated drug at 20,000 rpm for 1 h, at temperature 40°C using high speed cooling centrifuge (Andreas Hettich

GmbH and Co. KG, Tuttlingen, Germany). The supernatant was analyzed using UV- spectrophotometer (Shimadzu, Tokyo, Japan) to determine the fluconazole concentration based on a pre-established calibration curve.

#### In-vitro drug release from the optimized nanovesicular formulation

Drug release from the optimized nanovesicular formulations and the drug suspension was determined using the reverse dialysis technique in USP II dissolution apparatus (Pharm Test, Hainburg, Germany)<sup>25</sup>. The used dissolution medium was 900 mL phosphate buffer (pH 6.6). Dialysis bags (molecular weight cut off 12-14 kDa) was filled by 3 ml of the dissolution medium. The rotation speed adjusted to 50 rpm and the temperature set at  $37 \pm 1$  °C. Samples were taken at the following time intervals: 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8 and 24 h. The drug concentration analyzed spectrophotometrically at the predetermined  $\lambda_{max}$ .

PS values for the prepared fluconazole nanovesicles ranged between 450 and 753, as shown in Table 1.

#### Microbiological experiment

Different volumes (50  $\mu$ L, 100  $\mu$ L, 150  $\mu$ L) of each one of the 9 different nano forms of Fluconazole were added to 50  $\mu$ L of *Candida* suspension in three different tubes, mixed thoroughly and incubated for 2 minutes. 50  $\mu$ L is then taken from each mixture and dispensed to the plate of Sabouraud Dextrose agar and is then incubated at 37 °C for 48 hours. Similarly, the procedure is repeated with the normal form of the Fluconazole for the comparison of *Candida* growth, in addition to normal saline as negative control. Colonies then counted using colony

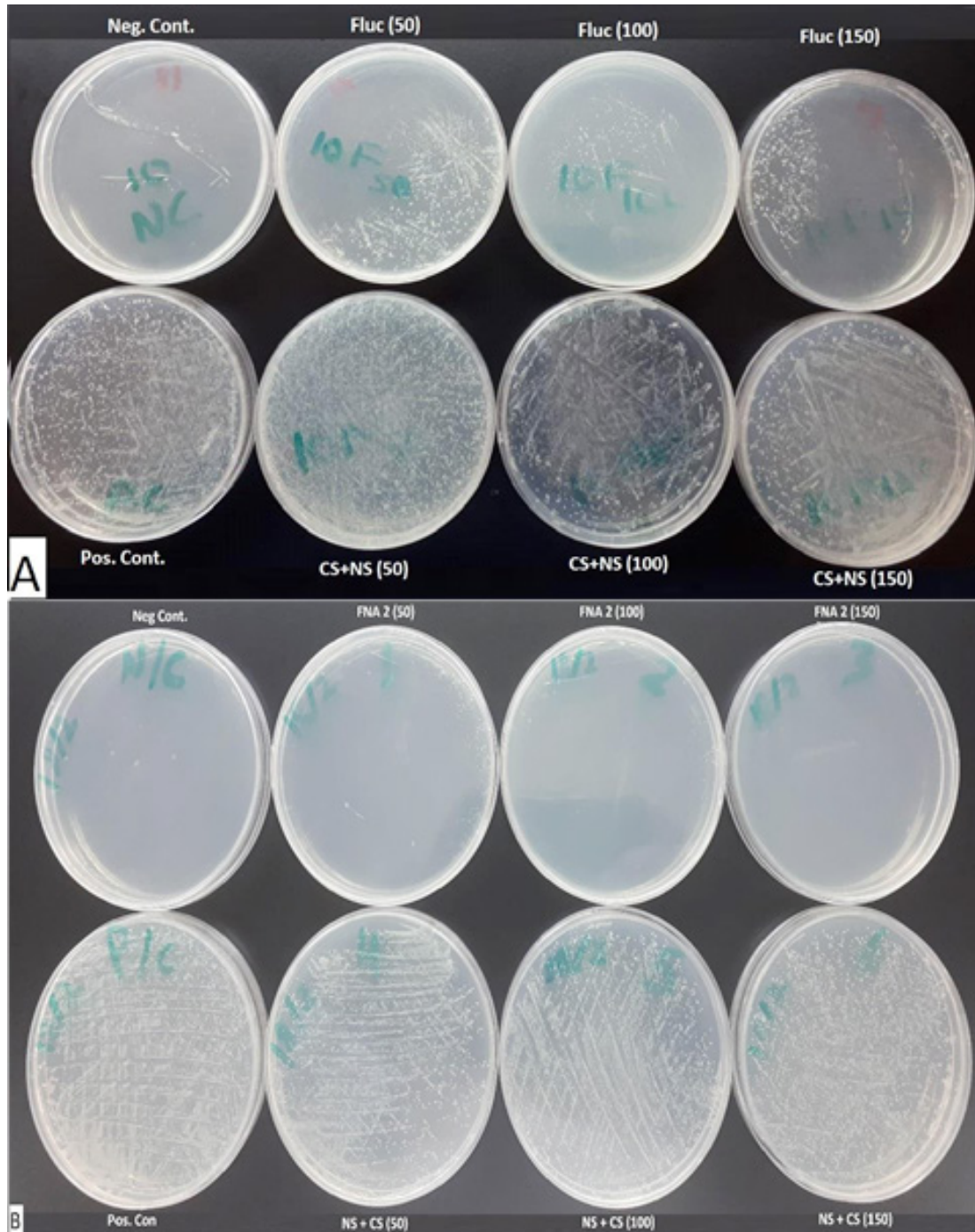
**Table 1.** Experimental runs, independent variables, and Measured responses of the central composite response surface experimental design for Fluconazole nanovesicular formulations

	Tween/Cetyl (T/C) ratio	Chitosan %	PS (nm)	PDI	ZP (mV)	EE (%)
F1	5	0.1	753.15 $\pm$ 6.24	1 $\pm$ 0.00	7.25 $\pm$ 0.68	96.55 $\pm$ 2.64
F2	5	0.2	693.08 $\pm$ 3.21	0.984 $\pm$ 0.04	-0.72 $\pm$ 0.24	94.53 $\pm$ 1.58
F3	5	0.3	723.47 $\pm$ 1.97	0.971 $\pm$ 0.05	3.63 $\pm$ 0.17	94.44 $\pm$ 3.05
F4	10	0.1	619.24 $\pm$ 9.04	0.617 $\pm$ 0.03	0.17 $\pm$ 0.06	93.711 $\pm$ 1.14
F5	10	0.2	681.65 $\pm$ 2.43	0.759 $\pm$ 0.04	-0.304 $\pm$ 0.03	92.49 $\pm$ 2.37
	10	0.2	684.17 $\pm$ 5.64	0.638 $\pm$ 0.02	0.143 $\pm$ 0.02	92.61 $\pm$ 1.45
	10	0.2	620.62 $\pm$ 1.67	0.691 $\pm$ 0.05	0.242 $\pm$ 0.06	92.00 $\pm$ 1.02
	10	0.2	651.90 $\pm$ 3.58	0.725 $\pm$ 0.03	0.531 $\pm$ 0.11	92.72 $\pm$ 0.08
	10	0.2	641.26 $\pm$ 7.69	0.677 $\pm$ 0.01	0.372 $\pm$ 0.25	92.92 $\pm$ 2.03
F6	10	0.3	689.33 $\pm$ 8.30	0.543 $\pm$ 0.03	-2.456 $\pm$ 0.07	92.25 $\pm$ 0.06
F7	15	0.1	480.35 $\pm$ 4.69	0.495 $\pm$ 0.02	2.256 $\pm$ 0.13	94.42 $\pm$ 2.41
F8	15	0.2	550.15 $\pm$ 1.83	0.152 $\pm$ 0.01	-2.21 $\pm$ 0.37	93.61 $\pm$ 0.09
F9	15	0.3	450.08 $\pm$ 7.66	0.339 $\pm$ 0.01	-0.465 $\pm$ 0.02	92.97 $\pm$ 1.73

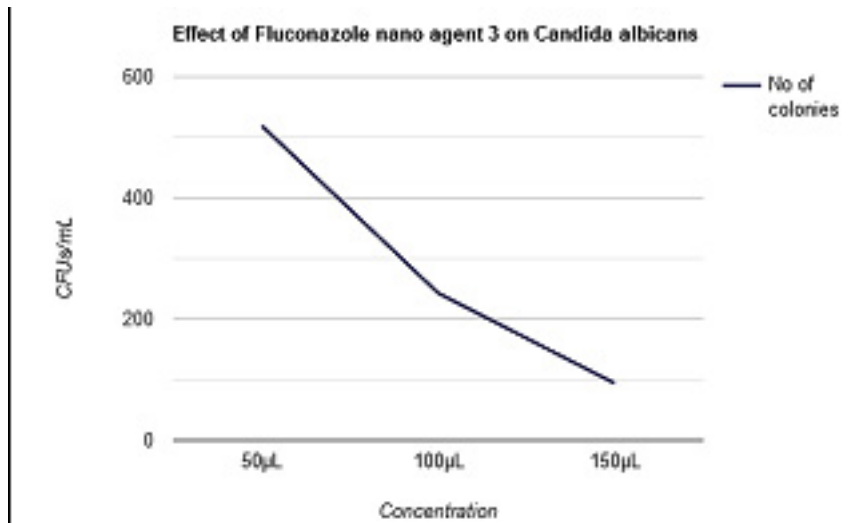
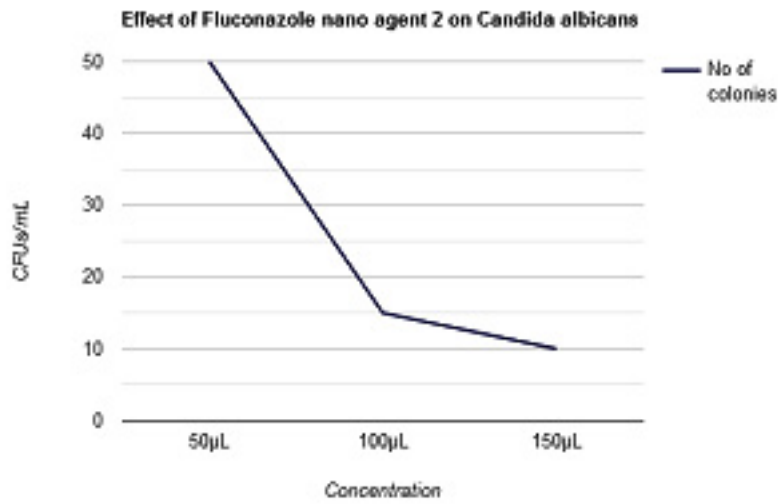
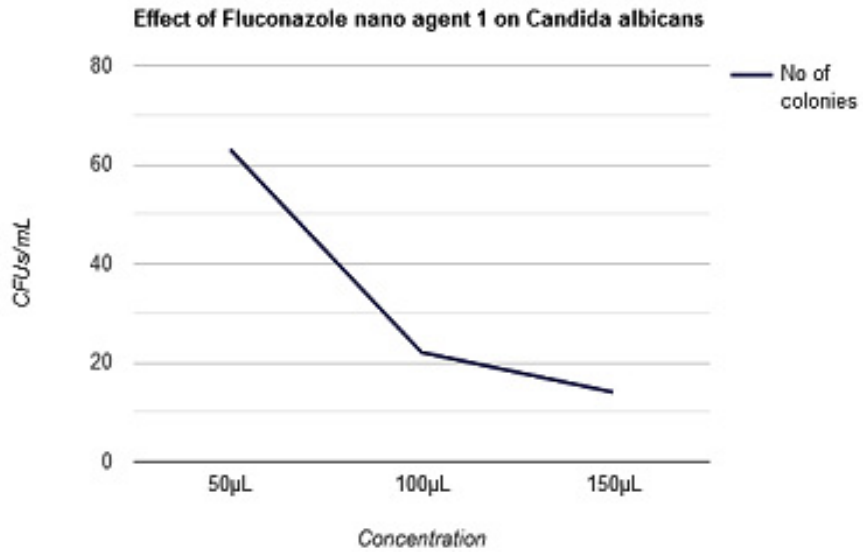
counter and the number of CFUs/mL plotted against concentration for different Nano forms of Fluconazole and difference has been estimated.

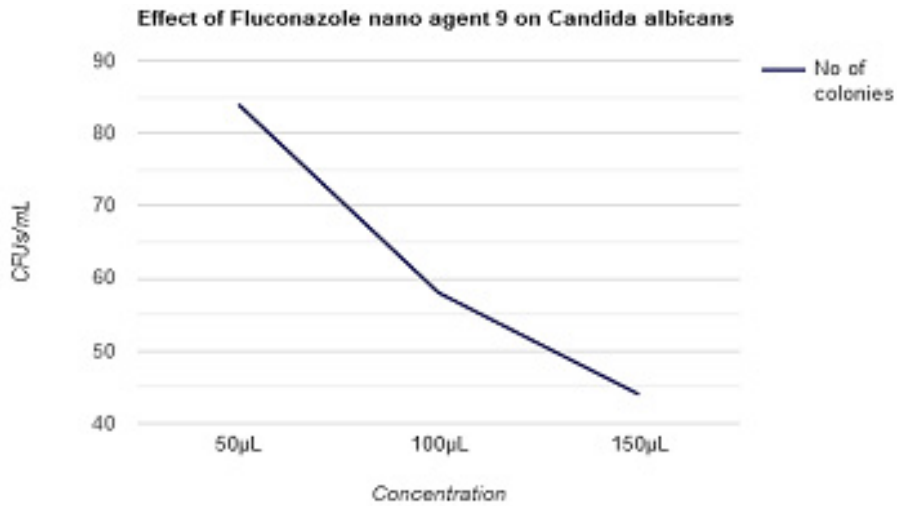
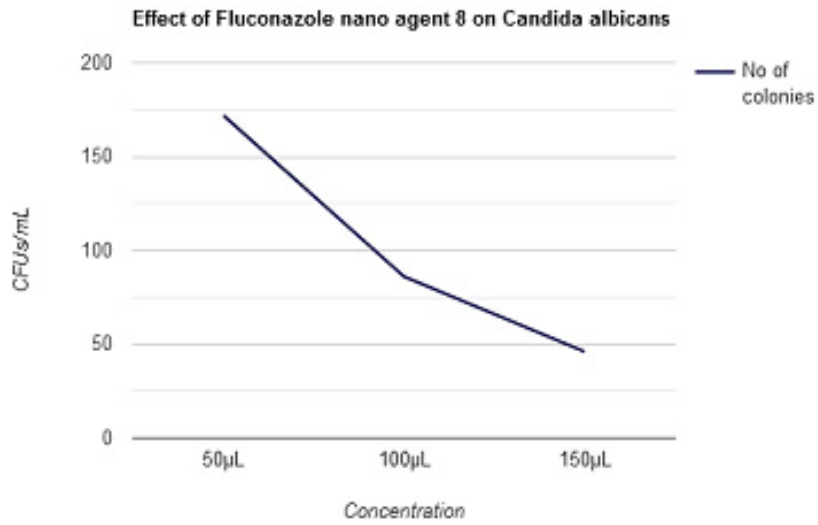
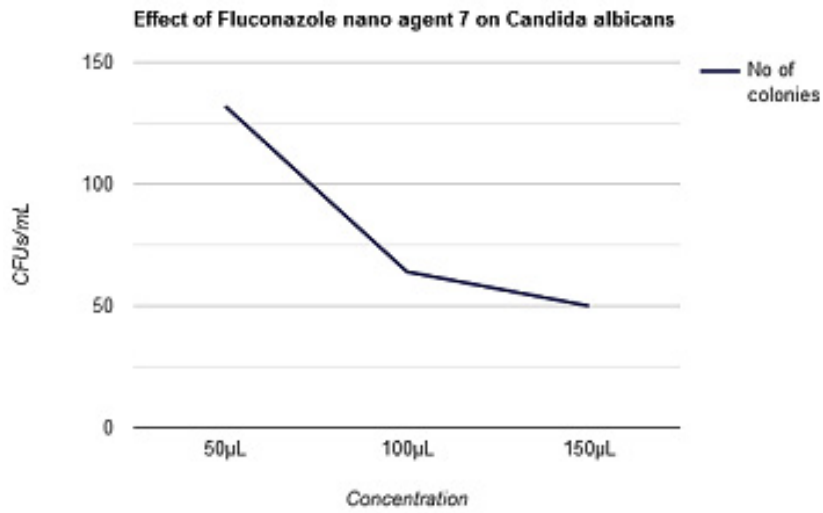
The colonies were enumerated using colony counter machine (Colony counter SC6

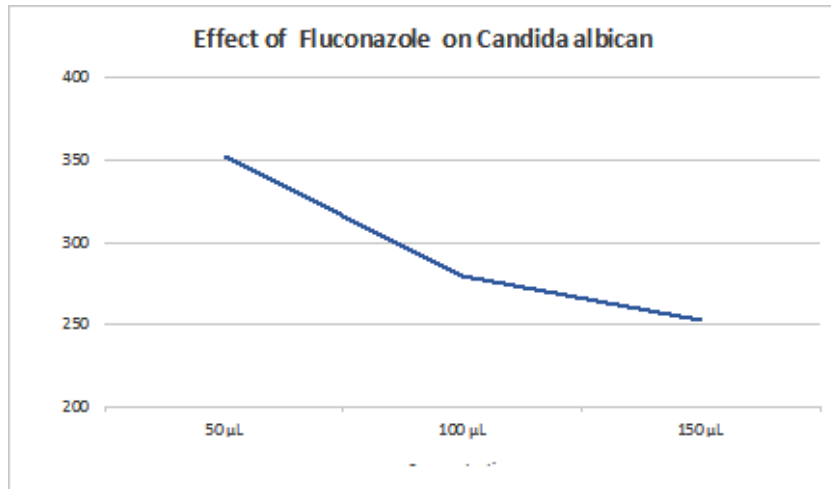
PLUS - Stuart) and the number of CFUs/mL were plotted against concentration for different Nano forms of the Fluconazole and the variation was estimated.



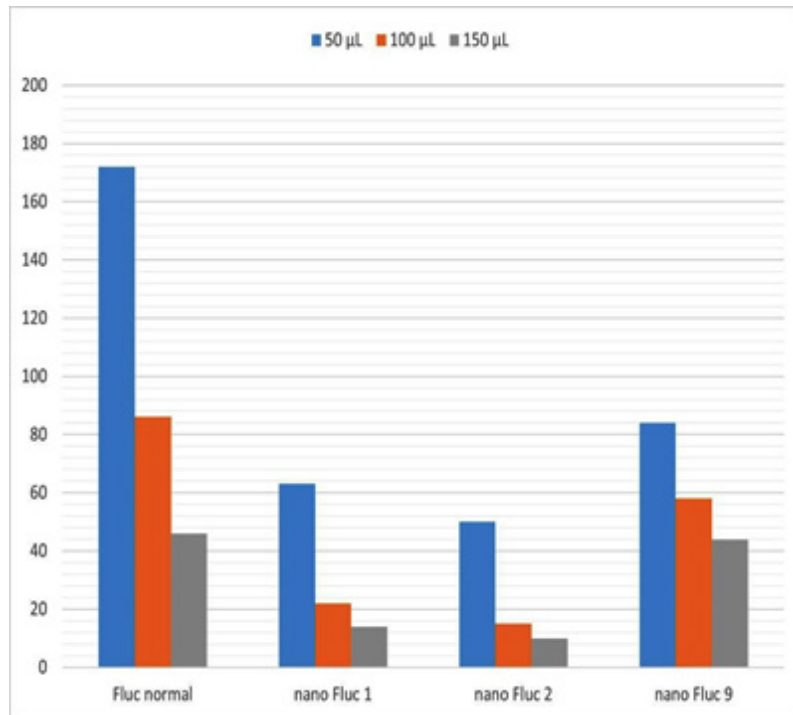
**Fig. 1.** Difference in colony count between Fluconazole (A) and Nano agents (B)







**Fig. 2.** Effect of Fluconazole and Nano agent 1,2,3,7,8,9 on *Candida albicans*



**Fig. 3.** Difference in colony count between the normal form of Fluconazole and Nano form 1, 2 and 9.

**RESULTS AND DISCUSSION**

The observed colony count shows a significant difference and increased effectiveness between the different nano forms in comparison to the normal form of Fluconazole as indicated in (figure 1). There is a significant decrease of colonies as the concentration of the nano agent

increased (table 1), the results indicate a significant difference among all the nine forms used in three different concentrations 50 µL, 100 µL and 150 µL after 48 hours of incubation at 37 °C. Nano agent 5 and 6 depict no significance in comparison to the normal form of Fluconazole (table 2. and 3). The maximum effect has been observed in the plate with highest volume 150 µL. The nano forms 1,

2, 3, 4, 7, 8 and 9 shows significant difference in comparison to the difference in concentration used to the normal form of Fluconazole (figure. 2) (table. 4). The colony count difference increases variably with increased concentration as seen in (figure 3).

Fluconazole (FLZ) is used to treat cutaneous fungal infections for more than 35 years<sup>6</sup>. FLZ has a moderately large molecular size and is hydrophobic, which promotes its absorption via intravenous or oral routes but makes it difficult to apply topically. In recent years, nano-based techniques for eliminating Fluconazole side effects and increasing medication efficiency have been investigated. The current overview examines nano-drug delivery technologies used to increase Fluconazole efficacy. Nanoparticles have

received renewed interest in recent decades for the management of fungal infections, resistance, and mutations<sup>17</sup>. The goal of this study was to create fluconazole nanoparticles (NPs) in various ratios to achieve the lowest particle size possible. The solvent evaporation technique was used to create nine formulas. The synthesized formula was then tested on *Candida albicans* positive strains, and the effect of each form was investigated further. According to the findings of the current study, Nano versions of Fluconazole are expected to be effective against pure strains of *Candida albicans*. Similar findings were obtained in study by Pandey and Ahmed<sup>1, 2</sup>.

Finding newer and more potent antifungal medicines to combat the resistant strains is required

**Table 2.** Comparison of colony count result yield from Normal Fluconazole and nano Fluconazole form (5)

Type of Fluconazole	50 µL	100 µL	150 µL
Fluconazole (NormalForm)	352	279	253
Fluconazole nano agent5	427	512	451
Difference in %	+21.315%	+83.51%	+78.26%

**Table 3.** Comparison of colony count result yield from Normal Fluconazole and nano Fluconazole form (6)

Type of Fluconazole	50 µL	100 µL	150 µL
Fluconazole (NormalForm)	352	279	253
Fluconazole nanoagent 6	426	310	300
Difference in %	+21.02	+11.11%	+47%

**Table 4.** Shows the decrease percentage of the colony count of the 9 forms of the nano fluconazole compared to the normal form except in number 5 and 6

Difference in %	50 µL	100 µL	150 µL
Nano 1	82.10%	92.11%	94.47%
Nano 2	85.8%	94.62%	96.05%
Nano 3	+47.4	12.9%	62.85%
Nano 4	29.31%	65.59%	68.83
Nano 5	+21.315%	+83.51%	+78.26%
Nano 6	+21.02	+11.11%	+47%
Nano 7	62%	77.06%	80.24%
Nano 8	51.4%	69.18%	81.82%
Nano 9	76.14%	79.12%	82.61%

as drug resistance develops. Azoles have a limited bioavailability because they are poorly soluble in water. In most cases, the drug is dispersed unevenly throughout the body, and certain cells participate in the drug's metabolism in the blood stream. Before use, the medication is only partially eliminated from the body. Antifungal agents have been modified thus far using a few procedures. Modern drug systems are now being produced and administered by new pharma firms. The most significant of these systems are hydrogels, nanofibers, nanoliposomes, niosomes, and nano-dendrimers, all of which are currently used frequently<sup>27</sup>.

Particle size and zeta potential range (753.15 ± 6.24 to 450.08 ± 7.66 nm) and (7.25 ±



0.68 to  $-0.465 \pm 0.02$  mV), respectively. Particle size and distribution width is often one of the most important quality-related parameters which affect other macroscopic properties of the nano-particle. Particles larger than 1  $\mu\text{m}$  and an increase in their number can show their physical instability<sup>26</sup>. Zeta potential is an important factor in determining the stability of the colloidal system and is the best indicator for determining the surface electric status of dispersions. In this study, the particle size of less than 1  $\mu\text{m}$  and zeta potential of ( $7.25 \pm 0.68$  to  $-0.465 \pm 0.02$  mV) indicated and confirmed the stability of the formulated nano- Fluconazole.

### CONCLUSION

The following conclusions may be drawn about the effect of each nano form of Fluconazole on *Candida albicans*. The nano forms of Fluconazole are estimated to work effectively against the pure strains of *Candida albicans*. With increased concentration of nano agent there is significant decrease in the growth of *Candida*. The small size of the nano agents therefore helps in better penetration of Fluconazole and proved to work effectively against the *Candida albicans*. The nano agents have shown significant variance in decreasing the colony of *Candida albicans* in comparison to the normal form of Fluconazole.

In conclusion we found that nano-fluconazole had a better affect than aqueous fluconazole. In light of these findings, the optimized nano vesicular formula could be considered as very promising nanocarriers for the application of fluconazole through increasing its antifungal effect.

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### Conflict of Interest

All authors confirmed there is no conflict of interest.

### Funding Source

NA.

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