

Evaluation Of Anti-Fungal Activity Of Solid Lipid Nanoparticles (SLN) Of Isavuconazole

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ABSTRACT

Human beings have always involved themselves in various activities to ensure their wellbeing and survival. In doing so, the human body has directed the release of different free radicals or reactive substances which are either inhaled or consumed. The research focused on assessing the antifungal efficacy of solid lipid nanoparticles (SLN) containing Isavuconazole. Isavuconazole was purchased from the (Sigma Aldrich, India). Fungus strains were obtained from the local microbiology and biochemistry laboratory, in Unnao. SLNs were prepared using spray drying method. In results, the highest zone of inhibition was determined against Rhizopus sp. as 7.61 mm, 6.03 mm, 6.58 mm, 7.20 mm, 5.14 mm, and 7.12 mm, in F1, F2, F3, F4, F5 and F6, respectively. It can be concluded that Solid Lipid Nanoparticles (SLN) of Isavuconazole are effective as anti-fungal agent against all the tested fungus strains. It suggests to confirm the mechanism of anti-fungal potential which might be through destruction of cell wall or mutations in the nucleotides or genetic materials. Further studies are suggested to determine the safety and efficacy of SLNs loaded with Isavuconazole, clinically....

Keywords: Solid lipid nanoparticles, Isavuconazole, anti-fungal activity, C. albicans, A. niger..

INTRODUCTION

Human beings have always involved themselves in various activities to ensure their wellbeing and survival. In doing so, the human body has directed the release of different free radicals or reactive substances which are either inhaled or consumed [1]. There are microorganism's bacteria everywhere. They are essential to maintaining our living environment. Worldwide, just a small percentage of micro-organisms cause disease and infection. These bacterial and fungal illnesses have a major effect on the health of the general people [2].

Since their inception in the early 1990s, solid lipid nanoparticles (SLNs) have established themselves as the most effective lipid-based colloidal carriers. This is among the most prevalent techniques employed to enhance the oral bioavailability of weakly water-soluble pharmaceuticals. Solid lipid nanoparticles are lipid constituents that are physiologically accepted and exist in a solid state at ambient temperature. They possess a submicron size spectrum of 50-1000 nm. The advantages of fat emulsions, liposomes, and polymeric nanoparticles are all integrated inside SLNs [3].

Isavuconazonium sulfate serves as the prodrug for the azole antifungal agent isavuconazole. Isavuconazonium is offered in both oral and intravenous forms and is swiftly transformed into the active isavuconazole by plasma esterases upon entering the systemic circulation. The dosage of isavuconazonium sulfate is 372 mg (corresponding to 200 mg of isavuconazole), given either intravenously or orally three times daily for 2 days as loading doses before initiating daily treatment with 372 mg [4].

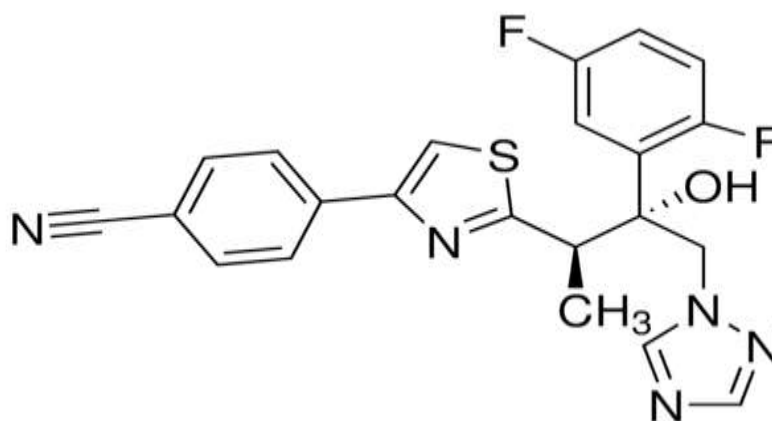


Fig 1. Structure of isavuconazole.

Molecular formula: $C_{22}H_{17}F_2N_5OS$

Molecular weight: 437.47

The dosage does not require modification for renal impairment or mild to moderate liver failure. Modifications might be necessary for considerable hepatic dysfunction. Nevertheless, explicit direction for individuals with significant liver impairment is presently absent. Similar to all azole antifungals, there is a possibility of hepatotoxicity [5]. The research focused on assessing the antifungal efficacy of solid lipid nanoparticles (SLN) containing Isavuconazole.

MATERIALS AND METHODS

Drugs and chemicals

Isavuconazole was purchased from the (Sigma Aldrich, India). Agar was purchased from the SD Fine Chemicals, Mumbai. Fungus strains were obtained from the local microbiology and biochemistry laboratory, in UP West.

Preparation of SLN [Spray-drying method]

Freitas and Mullera recommend use lipids with melting points over 70°C for spray drying. Isavuconazole was included and agitated until fully dissolved following the melting of the lipid constituents—stearic acid and cholesterol—at 60°C . Tween 80 and Span 20 were solubilized in distilled water to create an aqueous phase. Deionized water was subjected to heating while being continuously stirred to maintain a uniform temperature. The aqueous phase was blended with the lipid phase for 5 minutes at a velocity of 10,000 rpm using a homogenizer. Subsequently, a 25 Kw ultrasonicator was employed to sonicate the suspension for a duration of 5 minutes. The nanosuspension was ultimately brought down to ambient temperature [6].

Table 1. List of formulation ingredients

Formulation	Isavuconazole (mg)	Stearic acid (mg)	Cholesterol (mg)	Tweens 80 (mg)	Span 20 (%)	Deionized water (ml)
F 1	100	400	0.2	3400	2%	100
F 2	100	500	0.2	3400	2%	100
F 3	100	600	0.2	3400	2%	100
F 4	100	700	0.2	3400	2%	100
F 5	100	800	0.2	3400	2%	100
F 6	100	900	0.2	3400	2%	100

Evaluation of anti-fungal activity

The antifungal efficacy of SLN against fungi was determined by the well diffusion technique. Nutrient Agar broth was employed to nurture a 24-hour-old fungal culture, subsequently utilized to prepare a fungal suspension. Following sterilizing at 121°C (1.05 kg/cm² pressure) for a duration of 20 minutes, nutritional agar solution was included. A sterile spreader was employed to uniformly distribute the entire surface of the agar plates during the inoculation with 500 µl of each fungal suspension. Using a sterile cork borer, wells measuring 5 mm were created in the solidified medium, and each well was subsequently filled with SLN. The diameter (mm) of the inhibitory zone encircling the well was assessed following 24 hours of incubation at 30°C. Itraconazole gel was employed as a conventional antifungal preparation. All antifungal investigations were conducted in triplicate [7].

Fungus strains utilized for the screening of anti-fungal activity were as follows:

Aspergillus niger,

Candida albicans

Rhizopus species

Penicillium notatum,

RESULTS AND DISCUSSION

Total 6 formulations (F1-F6) of Solid Lipid Nanoparticles (SLN) of Isavuconazole were prepared using spray drying method. After characterization parameters, formulations were tested for anti-fungal activity using the various fungus strains.

Evaluation of anti-fungal activity

Formulation F1 showed the zone of inhibition as 6.13 mm, 5.29 mm, 7.61 mm, 6.42 mm against *A. niger*, *C. albicans*, *Rhizopus sp.* and *P. notatum*. Similarly, effective zone of inhibition was recorded against other fungus strains. However, zone of inhibition was determined highest against *Rhizopus sp.* as 7.61 mm, 6.03 mm, 6.58 mm, 7.20 mm, 5.14 mm, and 7.12 mm, in F1, F2, F3, F4, F5 and F6, respectively.

Moreover, standard formulation- Itraconazole gel showed the zone of inhibition as 6.71 mm, 7.56 mm, 8.91 mm and 6.40 mm against *A. niger*, *C. albicans*, *Rhizopus sp.* and *P. notatum*, respectively. Thus, Solid Lipid Nanoparticles (SLN) of Isavuconazole exhibited the anti-fungal potential against all the tested fungus strains which might be through breaking the cell wall or mutations in the nucleotides or genetic materials.

Table 2. Anti-fungal activity of Solid Lipid Nanoparticles (SLN) of Isavuconazole

Formulation	Zone of inhibition (mm)			
	<i>A. niger</i>	<i>C. albicans</i>	<i>Rhizopus sp.</i>	<i>P. notatum</i>
F1	6.13	5.29	7.61	6.42
F2	5.72	5.46	6.03	6.11
F3	5.31	4.17	6.58	5.66
F4	5.82	5.94	7.20	6.12
F5	6.04	6.25	5.14	5.72
F6	5.39	5.20	7.12	6.57
Itraconazole	6.71	7.56	8.91	6.40

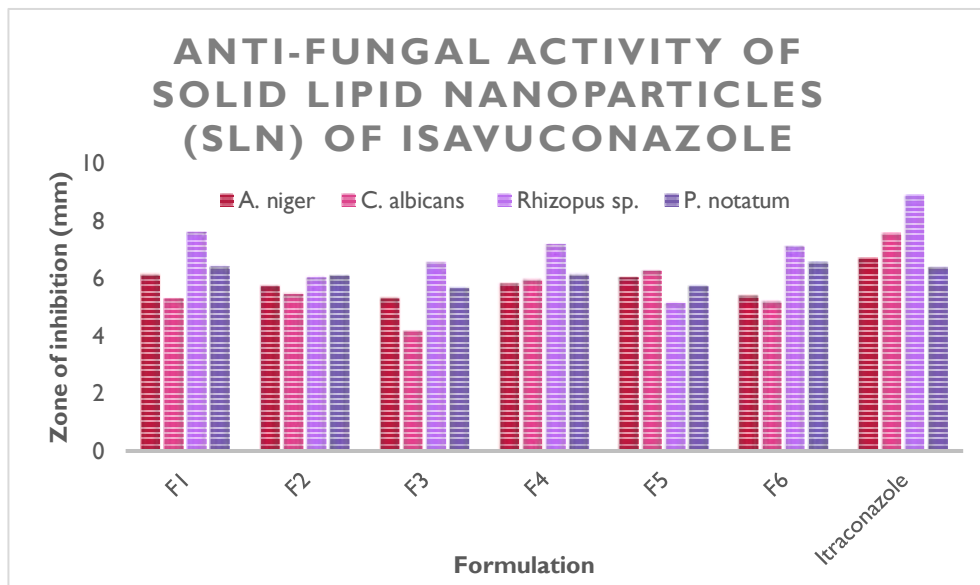


Fig 2. Graphical data of anti-fungal activity of Solid Lipid Nanoparticles (SLN) of Isavuconazole

CLSI M27-A3 and Etest results were compared for each antifungal agent and species as shown in Table 2. The essential agreement between the two methods was moderate and ranged from 52.7% (posaconazole) to 83.8% (fluconazole). According to the breakpoints adopted for *Candida* spp., the complete cohort of yeasts was classified according to the results of each test for fluconazole and voriconazole. Resistance to voriconazole was found in *R. mucilaginosa* (22.2%) and *Trichosporon* spp. [8]. All strains of *R. mucilaginosa* were fluconazole resistant. Appropriate first-line therapy for fungemia caused by *D. capitatus*, *S. cerevisiae*, *Trichosporon* spp., or *Rhodotorula* has not been defined, mainly due to the low number of cases reported; however, diminished susceptibility toward the most commonly used antifungal agents (amphotericin B and fluconazole) has been observed [9]. Isavuconazole has been shown to have good *in vitro* activity against *Aspergillus*, *Candida*, and *Cryptococcus* spp. Although the number of strains in the present survey is limited, the results corroborate previous reports [10] that isavuconazole is likely to be effective against infections caused by *D. capitatus*, *S. cerevisiae*, *Trichosporon* spp., or *Rhodotorula*. Agreement between the MIC results obtained by the two methods for the four triazoles tested was 83%. The principal discrepancies were observed for *R. mucilaginosa* (posaconazole and voriconazole). A definitive comparison between broth microdilution and Etest strips for these rare yeasts will require much larger numbers of clinical isolates [11][12].

Although mucormycoses (formerly zygomycoses) are relatively uncommon, they are associated with high mortality and treatment options are limited. isavuconazole is a novel, water soluble, broad-spectrum azole in clinical development for the treatment of invasive aspergillosis and candidiasis. The objective of this report was to collate data on the *In Vitro* activity of isavuconazole against a collection of 345 diverse mucorales isolates, collected and tested at eight study centers in Europe, Mexico and North America. Each study center undertook minimum inhibitory concentration (MIC) susceptibility testing of their isolates, according to EUCAST or CLSI guidelines. Across all study centers, isavuconazole exhibited MIC₅₀ values of 1-4 mg/L and MIC₉₀ values of 4-16 mg/L against the five genera. There were also marked differences in MIC distributions, which could be ascribed to differences in inoculum and/or endpoint. EUCAST guidelines appeared to generate modal MICs 2-fold higher than CLSI. These results confirm that isavuconazole possesses at least partial antifungal activity against Mucorales [13].

The outcomes of the disk diffusion assay indicate that *B. frutescens* evidently exhibits antibacterial characteristics against MRSA. *B. frutescens* appears to exhibit significant antibacterial efficacy against all gram-positive staphylococcal strains, suggesting that the plant extract targets the gram-positive cell wall, especially the staphylococcal cell wall, due to its considerably thicker peptidoglycan layer compared to gram-negative bacteria [14]. This external membrane consists of lipopolysaccharides that provide gram-negative bacteria with enhanced resistance to drugs that are unable to infiltrate it, such as glycopeptides like vancomycin [15]. The ethanol extract has demonstrated inhibitory growth in the highest zone against gram-positive bacteria such as MRSA (14.5 mm), *Staphylococcus aureus* (13 mm), and *Bacillus* (9.5 mm). The growth suppression shown considerable efficacy against the gram-negative bacteria *Escherichia coli* (8.5 mm) and *Klebsiella* (0 mm) [16].

CONCLUSION

Isavuconazole is a novel extended spectrum triazole with activity against yeasts, moulds, including Mucorales, and dimorphic fungi. Similar to other azoles, isavuconazole inhibits cytochrome P450 (CYP)-dependent 14 α -lanosterol demethylations, which is essential for fungal membrane ergosterol synthesis.

The highest zone of inhibition was determined against *Rhizopus sp.* as 7.61 mm, 6.03 mm, 6.58 mm, 7.20 mm, 5.14 mm, and 7.12 mm, in F1, F2, F3, F4, F5 and F6, respectively. It can be concluded that Solid Lipid Nanoparticles (SLN) of Isavuconazole are effective as anti-fungal agent against all the tested fungus strains. It suggests to confirm the mechanism of anti-fungal potential which might be through destruction of cell wall or mutations in the nucleotides or genetic materials. Further studies are suggested to determine the safety and efficacy of SLNs loaded with Isavuconazole, clinically..

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