Formulation and Evaluation of Itraconazole Nanoemulsion for Enhanced Oral Bioavailability
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Purpose
Over the last two decades fungal infections have been increased among patients going through organ transplant, or receiving chemotherapy and due to aggressive use of broad-spectrum antibiotics. Itraconazole (ITR), a broad-spectrum potent antifungal agent has poor bioavailability because of low aqueous solubility. Presently, available in the form of capsule (Sporanox®, Itaspore®, Canditral®) and solution dosage form (Sporanox oral solution) contains high amount of solubility enhancing agents such as PEG 20000 and HP-β-cyclodextrin, which causes osmotic diarrhea. Various techniques for enhancement of solubility of ITR have been reported however they have some inherent limitations. Hence, there is need of an approach, which is safe, simple, economic and effective in improving the oral bioavailability of such drug. The present investigation aimed at development of Itraconazole loaded nanoemulsion (NE) to enhance its oral bioavailability.

Methods
Oil phase was selected by determining the solubility of ITR in various oils. Selection of surfactant and co-surfactant was carried out based on stability of emulsion observed over a period of 15 days. In present investigation 3A3 full factorial design was assessed between 3 levels using 3 independent factors namely surfactant concentration (% w/v) (X1), speed of stirring (rpm) (X2) and sonication amplitude (% amplitude) (X3). Drug entrapment (%) (Y1) and globule size (nm) (Y2) were taken as dependent variables. ITR loaded nanoemulsion was prepared by high speed stirring followed by probe sonication to obtain nano size. Formulation was visually observed for creaming, cracking and phase separation. The mean Globule size, zeta potential and Polydispersity index were measured by dynamic light scattering using Malvern Zetasizer. Morphology and structure of the nanoemulsion were studied using Transmission Electron Microscopy. In Vitro diffusion studies were performed for both, nanoemulsion as well as plain drug suspension (control). Ex Vivo gastro intestinal permeability study was done in rat stomach and intestine. Laser scanning confocal microscopy study was done in rat intestine to study the permeation of formulation. Antimycotic study was performed using Agar-cup diffusion method using the culture of Aspergillus niger, to compare the efficacy of ITR plain drug suspension, ITR Nanoemulsion and Placebo Nanoemulsion. Pharmacokinetic study for plain drug suspension and ITR Nanoemulsion was performed in healthy male Wistar rats and the parameters, namely, Cmax, Tmmax, t ½ and AUC 0-48h, were calculated using non-compartmental analysis method for each plasma concentration-time point. Short term accelerated stability studies were carried out at two different storage conditions i.e. under refrigerated condition (2-8°C) and at room temperature (25 ± 2°C) for a period of three months.

Results
The results of solubility study of ITR in different oils showed that the drug has highest solubility in Capmul MCM C8 and was thus selected as oil phase. Cremophor EL and Pluronic F127 were selected as surfactant and co surfactant based upon the stability they conferred upon the nanoemulsion. Globules were of 100.9 nm size with zeta potential of -35.9 ± 1.2 mV. Results of TEM analysis were in agreement with Dynamic Light Scattering and showed spherical globules showing no signs of agglomeration. The results of 3A3 full factorial design analyzed by Design Expert Ver. 7.0.0, showed that surfactant concentration has maximum positive effect on entrapment efficiency, followed by stirring speed and lastly sonication amplitude. Increase in surfactant concentration and sonication amplitude decreased the globule size while increase in stirring speed slightly increased the globule size. In-vitro diffusion study showed that the total percentage diffusion was higher for the nanoemulsion (94.87%) than for the plain Itraconazole suspension (57.14%). Similarly, Ex Vivo study depicted that the drug diffusion from both the rat stomach as well as intestine was significantly higher for the NE as compared to the ITR plain suspension and ITR marketed preparation (Sporanox®). The Laser Scanning Confocal Microscopy corresponded well with the data obtained by ex vivo permeation study, as high fluorescence is seen distributed throughout the intestinal membrane showing good penetration of the formulation. Antimycotic study confirmed the retained efficacy of ITR in nanoemulsion showing higher zone of inhibition than plain drug and placebo NE. AUC0-48h of ITR NE was found to be 13470.53 (ng/ml*h) while that of plain drug suspension was 5504.03 (ng/ml*h) indicating a two-fold increase in the bioavailability of Itraconazole. The prepared Nanoemulsion was stable at both, refrigerated and room temperature conditions.

Conclusion
In present investigation an attempt was made to curtail the problems associated with poor bioavailability of ITR by preparing stable nanoemulsion. The prepared NE was efficacious than plain drug as well as marketed formulation as confirmed by various studies and showed better penetration through intestinal membrane. Pharmacokinetic parameters showed that nanoemulsion of ITR seem to be a promising formulation for enhancement of its oral bioavailability.