Tailoring thixotropic mixed-lipid nanoc-onstructs of voriconazole for the management of Vulvovaginal candidiasis: Formulation, statistical optimization, *in vitro characterization and in vivo assessment*

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ABSTRACT

Vulvovaginal candidiasis is a pervasive gynecological condition among women worldwide due to infection recurrence and resistance to conventional drugs. This calls for a novel formulation of alternative medication with enhanced efficacy. This study aimed to fabricate mixed lipid nanoconstructs (MLNCs) of voriconazole (VCZ) with a low concentration of lipids applying high shear homogenization and ultrasonication to form a semisolid formulation. Tefose 63 and Gelot 64 were employed as emulsifiers special for vaginal preparations; for their mucoadhesive properties and their texture enhancing characters, although usually used as lipids in different lipid carriers. A 2^4 factorial design was established, and the optimized formulation was prepared using 10% total lipids, in which solid lipids (Sterotex NF: Glyceryl monostearate) ratio was 1.92:1 and the oils percentage was 30% (Maisine: Glyceryl monooleate, in the ratio of 1:1), and the emulsifiers mixture (Tefose 63: Gelot 64) ratio was 1:1, as 10% of total formulation weight. The optimized formulation with a viscosity of 964.49 ± 57.99 cp showed spherical nanoparticles (322.72 ± 15.11 nm) that entrapped 67.16 ± 3.45% of VCZ and exhibited release of 70.08 ± 2.87% in 8 h. The optimized formulation with high bioadhesive potentials significantly reduced the fungal burden in female Wistar rats infected with vaginal candidiasis, compared to the aqueous VCZ suspension ($p < 0.05$). Furthermore, in vivo histopathological findings proved the effectiveness and the safety of the optimized MLNCs formulation after vaginal application. Inclusively, MLNCs formulation could be a promising vaginal delivery system of VCZ for the treatment of vulvovaginal candidiasis.

OBJECTIVES

- The possibility of formulating VCZ loaded mixed-lipid nano-constructs (MLNCs) with a low lipid concentration and emulsifiers specifically used in vaginal preparations and known for their mucoadhesive properties, customizing high shear homogenization and ultrasonication to form a vaginally applicable dosage form with semisolid consistency, for the treatment of VVC.
- In *vivo* study were conducted to assess the efficacy and the safety of the optimized formulation in rats infected with vaginal candidiasis.

MATERIALS & METHODS

Table 1: Composition of the prepared VCZ- MLNCs

#	Factors			
	A Total Lipids (%)	B Sterotex NF: GMS	C Tefose 62: Gelot 64	D Oils in lipid phase (%)
MLNCs 1	10	1:1	0:1	10
MLNCs 2	10	1:1	0:1	30
MLNCs 3	10	1:1	1:1	10
MLNCs 4	10	1:1	1:1	30
MLNCs 5	10	2:1	0:1	10
MLNCs 6	10	2:1	0:1	30
MLNCs 7	10	2:1	1:1	10
MLNCs 8	10	2:1	1:1	30
MLNCs 9	20	1:1	0:1	10
MLNCs 10	20	1:1	0:1	30
MLNCs 11	20	1:1	1:1	10
MLNCs 12	20	1:1	1:1	30
MLNCs 13	20	2:1	0:1	10
MLNCs 14	20	2:1	0:1	30
MLNCs 15	20	2:1	1:1	10
MLNCs 16	20	2:1	1:1	30

RESULTS & DISCUSSION

Figure 1: TEM micrographs of the optimized VCZ loaded

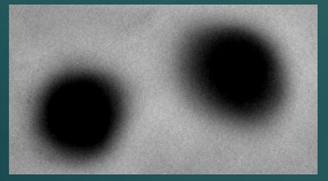
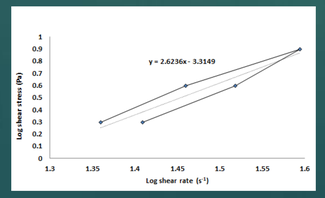


Figure 2: The rheogram of the optimized VCZ loaded MLNCs formulation showing the thixotropic behavior of the formulation



RESULTS & DISCUSSION

Figure 3: Pareto charts demonstrating the effect of the factors; A: Total lipids percentage, B: The solid lipids mixture ratio C: the oils (Glyceryl monooleate (GMO) and maisine, in ratio 1:1) percentage in lipid phase, and D: The emulsifier mixture ratio (Tefose 63: Gelot 64) on (a) (EE %), (b) (PS), (c) viscosity and (d) the cumulative amount of drug released after 8 h (Q 8).

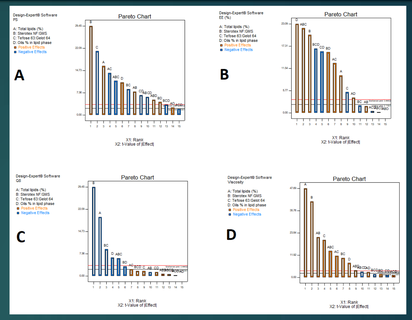
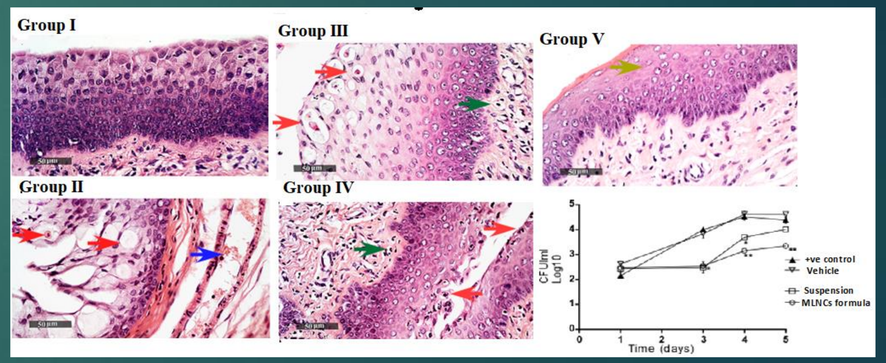


Figure 4: Microscopical histopathological examinations of rats with vaginal candidiasis 7 days after infection stained by Hematoxylin and Eosin.
I. Section of negative control rat vagina showing normal morphological features of the vaginal mucosa with almost intact lining epithelium. II. Section of positive control rat vagina showing apoptotic vaginal keratinocytes (red arrow), intraluminal desquamated epithelial cells (black arrow), congestion and dilatation of submucosal blood vessels (blue arrow) as well as subepithelial inflammatory cells infiltrate (green arrow). III. Section of rat vagina treated with the optimized drug-free MLNCs showing the same records as the positive control samples. IV. Section of rat vagina treated with VCZ suspension showing degenerative changes in superficial layers of vaginal keratinocytes (red arrow) with occasional intraluminal desquamated cells (black arrow), subepithelial inflammatory cells infiltrates (green arrow), and minimal records of congested submucosal blood vessels. V. Section of rat vagina treated with the optimized VCZ loaded MLNCs showing normal morphological features of the vaginal mucosa with almost intact lining epithelium (yellow arrow) and nominal congested submucosal blood vessels (blue arrow).



CONCLUSIONS

MLNCs were successfully formulated using low lipids concentrations, consisting of mixtures of solid lipid and oils, applying high shear homogenization and ultrasonication. MLNCs were stabilized by Tefose 63 and Gelot 64. A full 2 ^{4}Factorial design was exploited for the optimization of MLNCs. The optimized formulation displayed nano-spherical particles, with high VCZ EE%, and showed thixotropic consistency with high bioadhesivity. The in vivo studies revealed that the incorporation of VCZ into MLNCs reduced the fungal burden, in female Wistar rats infected with vaginal candidiasis, compared to the aqueous VCZ suspension ($p < .05$). Furthermore, the histopathological findings proved the effectiveness of the optimized formulation in the management of VVC. Consequently, the obtained results suggest that the formulated dosage form can be considered as a potential nanoscaled lipid-based carrier for improving the delivery of VCZ through the vaginal mucosa. Further clinical studies are planned to authenticate the effectiveness of the optimized formulation in the management of VVC.