English abstract

The *in vitro* antifungal activity of nano-liposomal amphotericin B against common and rare fungal pathogens causative of non-dermatophyte dermatomycosis

Background and objective: Pathogenic or opportunistic fungi, including molds, yeasts, and dimorphic fungi induce cutaneous and subcutaneous fungal infections. The incidence of fungal infections resistant to common skin treatments is increasing worldwide at an alarming rate, posing a major challenge to health professionals. Nanoliposomes can enhance penetration through the stratum corneum, reduce the side effects of systemic drugs by their local application, and overcome many barriers to dermal drug delivery. Therefore, our study was defined to determine the *in vitro* antifungal activity of nanoliposomal amphotericin B against common and rare fungal pathogens to obtain sufficient and required information regarding the introduction and efficacy of the new drug nanoliposomal amphotericin B.

Methods: The experimental study was conducted over 30 months from September 2021 to April 2024 at the Molecular Biology Laboratory of Medical Mycology, Tehran University of Medical Sciences, on 136 clinical fungal isolates. Nanoliposomes were prepared using phosphatidylcholine, cholesterol, and amphotericin B using the ethanol injection method. The morphology, size, and surface charge of the developed nanoliposomal amphotericin B drug were measured by transmission electron microscopy (TEM) and Zeta analyzer, respectively. The Antifungal susceptibility testing (AFST) to nanoliposomal amphotericin B, amphotericin B, fluconazole, itraconazole, clotrimazole, and terbinafine on clinical yeast isolates of candidiasis agents (42 isolates), rare yeasts like (6 isolates) by microdilution method based on CLSI M27-4th ed standard method and string clinical isolates of agents of mucormycosis (46 isolates), aspergillosis (24 isolates), fusariosis (6 isolates), myestoma (3 isolates), chromoblastomycosis (3 isolates) and rare string isolates (5 isolates) based on the CLSI standard method M38-3th ed. done both methods were used to investigate the dimorphic isolation of *Sporothrix shenkei* in two yeast and mold phases.

Results: Nanoliposomal amphotericin B nanoparticle size was 113.5 ± 10.4 nm and its morphology was round. Also, the zeta potential of amphotericin B nanoliposomal particles was -36.5 ± 4.2 mV. In evaluating the AFST of yeast isolates, 10% to amphotericin B, 57% to fluconazole, 52% to itraconazole, 86% to clotrimazole, and 48% to terbinafine were resistant, and 100% of yeast isolates were sensitive to nanoliposomal amphotericin B with a minimum

inhibitory concentration range of 0.062-2 µg/ml and a geometric mean range of 0.19-0.54. In evaluating the AFST of filamentous isolates, 50% of *Rhizopus oryzae*, 55% of *Aspergillus flavus*, 62.5% of *Aspergillus fumigatus*, 33% of *Pseudallescheria boydii* compared to itraconazole, 100% of *Pseudallescheria boydii*, 2.4% of *Rhizopus oryzae* compared to amphotericin B were non-wild type, while 97% of filamentous isolates were wild type strains to nanoliposomal amphotericin B with a minimum inhibitory concentration range of 0.015-4 µg/ml and a geometric mean range of 1.08-0.09.

Conclusion: The present study showed that the nanoliposomal amphotericin B drug against yeast and filamentous fungal agents with a sensitive and resistant drug pattern causing candidiasis, trichosporonosis, geotrichosis, mucormycosis, aspergillosis, fusariosis, chromoblastomycosis, pheohyphomycosis and sporotrichosis has stable and effective *in vitro* performance. which can promise an effective treatment alongside current common treatment.

Keyword: Amphotericin B nanoliposome; Azoles; Dermatomycosis; Drug resistance