Antifungal Potential of Nanostructured Usnic Acid and its Association with Fluconazole against Candida albicans

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Candidiasis is an infection can affect the skin, mucous membranes, and internal organs occurring more frequently in immunocompromised individuals, antibiotic users, or those with microbiota imbalances, and may be associated with high morbidity and mortality rates, especially when caused by fluconazole-resistant *Candida albicans*. In this context, nanotechnology emerges as a promising therapeutic alternative, allowing the development of controlled-release systems containing natural bioactives with limitations such as low water solubility and high toxicity. Usnic acid (UA), a natural molecule with well-described biological properties, stands out in this regard; however, it exhibits hydrophobic characteristics and high hepatotoxicity. Therefore, the encapsulation of UA in chitosan-coated zein nanospheres (UA-ZNPs-CH) and/or its association with fluconazole (FLU) presents itself as a promising innovative strategy to confer greater stability, efficacy, bioadhesiveness, and enhance antimicrobial action, potentially promoting the resensitization of resistant strains. Thus, the present study aimed to evaluate the antifungal potential of AU-ZNPs-CH in association with FLU against fluconazole-sensitive or resistant *C. albicans.* AU-ZNPs-CH were prepared using the solvent displacement method followed by evaporation, and characterized in terms of particle size (PS), polydispersity index (PDI), zeta potential (ZP), pH, and encapsulation efficiency (%EE). The minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) were determined using the Clinical and Laboratory Standards Institute (CLSI) microdilution method, and the interaction between AU-ZNPs-CH and FLU against a clinical isolate of FLU-resistant *C. albicans* was performed using the Checkerboard method. The AU-ZNPs-CH sample showed PS of 296.9 nm, PDI of 0.16, ZP of +51.1 ± 1.0 mV, pH of 4.2, and %EE of 92.65%. The MIC and MFC of AU was >125 g/mL for all strains used in this study, while for AU-ZNP-CH, the MIC was 31.25 µg/mL for URM 9169 and URM 5393, and 15.6 µg/mL for CA 5039 and URM 4986. The MFC of AU-ZNP-CH was 125 µg/mL for CA 5039, URM 9169 and URM 5393, and 62.5 µg/mL for URM 4986. In vitro interaction analysis between AU+FLU and AU-ZNP-CH+FLU was performed on strain CA 5039, *C. albicans* resistant to FLU, revealing ICIF values between 0.04 and 0.09, characterizing synergistic interactions. The results obtained demonstrate that nanoencapsulation of AU promoted substantial improvements in its physicochemical and biological

properties, mainly reflected in the high encapsulation efficiency, the particle size suitable for oral administration, and the zeta potential, which indicate good colloidal stability and bioadhesive potential due to the chitosan coating, a relevant aspect for interaction with fungal cell surfaces. The significant reduction in the MIC and MFC of AU after encapsulation evidenced the increase in antifungal efficacy, possibly related to greater apparent solubility and better interaction with the fungal cell. The strong synergistic interaction observed between AU-ZNPs-CH and FLU is also noteworthy, indicating potentiation of antifungal activity, but also a possible mechanism for resensitization of fluconazole-resistant strains. In conclusion, nanostructured systems play a promising role as an innovative strategy to overcome the pharmacological limitation of bioactive compounds and combat antifungal resistance.

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