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Design and Evaluation of Nanoencapsulated Oregano Essential Oil as Alternative Treatment to Candida Albicans Infection

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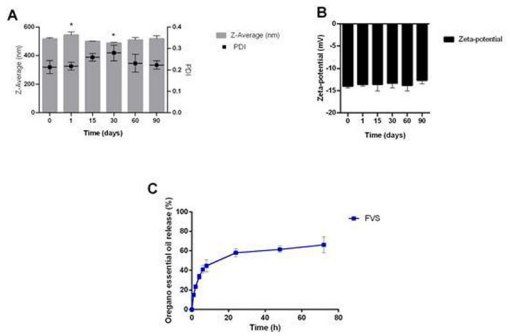
Objectives Vulvovaginal candidiasis (VVC) is characterized as a very common fungal infection with a huge negative impact on women's health worldwide. The limited effective and safe therapies available and the consequent increase in resistance to antifungal agents, make the development of new fundamental therapies crucial. Natural products, such as essential oil (EOs), are currently being evaluated regarding their antimicrobial activity. However, the EOs effect depends on several factors such as photosensitivity, high volatility, low water-miscibility, and degradability when exposed to temperature, decreasing their bioavailability. To overcome these limitations, micro or nanoencapsulation has emerged as an efficient technique to protect and control the release of EOs, improving the water-solubility and bioavailability of lipophilic compounds. Thus, the main goal of this study was to produce and characterize nanoparticles of keratin (KNP´s) loaded with oregano essential oil (OO-KNP´s) as an alternative treatment for VVC.

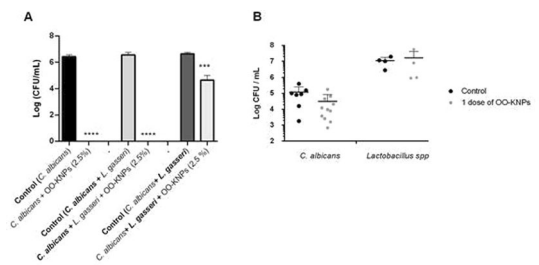
Materials and methods The OO- KNP's were produced by ultrasound cycles through a high-intensity ultrasonic and characterized regarding morphological and physicochemical parameters (particle stability, OEO encapsulation efficiency and release profile). First, the OO-KNP´s effect against *C. albicans* in vitro was evaluated by broth microdilution and diffusion in agar. The activity against biofilm was quantified by colony forming units' enumeration (CFUs). Then, the efficacy of OO-KNP's on in vivo VVC mouse model was also studied. For this, 20 female BALB/C female mice (18.7 ± 1.2 gr of weight) were infected with 1.33×10^8 CFU/mL of *C. albicans* and 24 h after the infection, 11 animals recived single dose of OO-KNP's intravaginally and the rest of the animals recived saline solution, remaining as controls. Vaginal fluid were collected in all the animals 24 h and 48 h after the treatment to quantify *C. albicans* and *Lactobacillus* species growth in culture medium (CFUs/mL).

Results The OO-KNP´s remained stable over time and exhibited high encapsulation efficiency (99.42%). Furthermore, a controlled rate of OEO release was also observed during the first 24 h in the synthetic vaginal fluid, due to the destabilization of the particles in this medium. The size of these particles, approximately 500 nm, which is suitable for penetration of delivery systems inside fungal cells. In fact, a total inhibition of the planktonic growth of *C. albicans* was obtained. Furthermore, the results showed that, in vitro, the application of only 2.5% OO-KNP´s eradicates mature *C. albicans* biofilms while preserving the *Lactobacillus* species. In in vivo, a single intravaginal application of OO-KNP´s induced a reduction of *C. albicans* growth (0.6 Log CFU/mL). Furthermore, one of the most important factors, this therapy keeps intact the remaining microflora in relation to the *Lactobacillus* species, confirming previuos in vitro results.

Conclusions The stability of OO-KNP´s over time and its effect, in vitro, against *C. albicans* infection was verified. Despite the need to complement our in vivo study, our preliminary results showed that this new OEO therapeutic approach can be a promising alternative or complementary therapy for the treatment of VVC. In addition, OO-KNP´s may have a less harmful effect on women's health, due to their natural characteristics.



Characterization of nanoparticles of keratin loaded with oregano essential oil (OO-KNP´s). (A) particle size (Z-average) and polydispersity (PDI); * indicate statistical difference in particle size when compared to the results obtained at time 0 (* p< 0.05); (B) surface charge (æ-potential) and (C) *In vitro* release profiles of oregano essential oil from keratin-based particles in simulated vaginal fluid, over 72 h.



Effect of the nanoparticles of keratin loaded with oregano essential oil (OO-KNPs) on infection of *Candida albicans* and the induced effect on *Lactobacillus* species. (A) I*n vitro* assay; (B) *In vivo* assay. * indicate statistical difference in comparison with respective control (*** p<0.001, **** p< 0.0001).