

**TITLE:** ANTIMICROBIAL EFFECTS OF NITRO-CHALCONE AGAINST MIXED BIOFILMS OF *CANDIDA ALBICANS* AND *STREPTOCOCCUS MUTANS*, AND ITS TOXICITY IN *GALLERIA MELLONELLA*

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**ABSTRACT:** Microbial biofilms are etiological factors of several oral diseases, such as carie and periodontal disease, and many of these biofilms functions as mechanisms of virulence, which encourages the search for novel compounds capable of inhibiting their growth in biofilms and virulence. In this study we investigated the antifungal activity of nitro-chalcone (NC-E08) against *Candida albicans* MYA 2876 and *Streptococcus mutans* ATCC 700610, and further determined its toxicity *in vivo* using *Galleria mellonella* model. The Minimum Inhibitory and Bactericidal/Fungicidal Concentrations (MIC/MFC/MBC) of NC-E08 were determined as well as its effects in the formation and morphology (SEM) of mature mono-species and mixed biofilms of *C. albicans* and *S. mutans*. The data were analyzed by ANOVA with Tukey's post-hoc test ( $p < 0.05$ ). NC-E8 showed excellent antifungal activity, with MIC of 15.6  $\mu\text{g/ml}$  and MFC of 62.5  $\mu\text{g/ml}$  on *C. albicans*, and MIC of 3.9  $\mu\text{g/ml}$  and MBC of 31.25  $\mu\text{g/ml}$  on *S. mutans*. Treatment with NC-E08 at 10xMIC reduced *C. albicans* mature biofilm survival and formation by 55% and 0%, respectively. For *S. mutans*, treatment with NC-E08 reduced biofilm survival and formation by 38% and 42.5%, respectively. There was a reduction in survival of treated mixed mature biofilms of 41% and 46% for *C. albicans* and *S. mutans*, respectively, while mixed biofilm formation was reduced by 50% in *S. mutans* and totally inhibited in *C. albicans*. The compound significantly affected the biofilm architecture/structure under all conditions analyzed, while showing negligible toxicity in *G. mellonella* larvae at 10xMIC. In conclusion, NC-E08 reduces both mature and forming biofilms, with low toxicity *in vivo*. This nitro-chalcone could be considered a promising molecule for the prevention and treatment of mixed infections caused by *S. mutans* and *C. albicans* biofilms.

**Keywords:** Biofilms, *S. mutans*, *C. albicans*, toxicity

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