

## Anti-adhesive and anti-biofilm activity of NC-E08. *In vitro* and *in vivo* study using *Galleria mellonella* infection model

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### Abstract

Biofilms are organized microbial communities formed from an ecological succession. Biofilm formation functions as a mechanism of virulence and favors the development of diseases, including oral diseases such as dental caries and periodontal disease, in which the microorganisms *Streptococcus mutans* and *Candida albicans* are closely related. Previous studies have shown that interactions between *S. mutans* and *C. albicans* are associated with the pathogenesis of early childhood caries (ECC). Therefore, there is a great interest in finding new prototypes for antimicrobial drugs, mainly for the development of structural analogues of chalcones, which constitute one of the largest classes of natural products belonging to the flavonoid family and are considered strategic molecules for this purpose.

### Key words:

Dual biofilms, toxicity, nitro-chalcone.

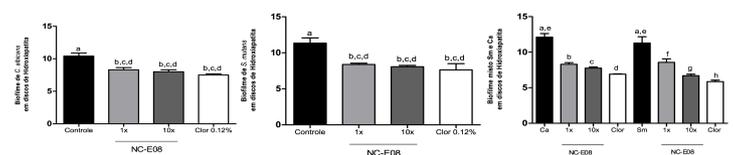
### Introduction

Biofilms are associated with many infectious diseases in humans, including those occurring in the mouth. *S. mutans* can efficiently cope with environmental stresses, which contributes to its ability to establish biofilms, to persist in the host, and to compete with other oral bacteria, particularly when conditions are conducive to initiation and progression of dental caries (Seneviratne et al., 2011). Interestingly, results from several clinical studies reveal that *C. albicans* is often detected in high numbers with *S. mutans* in biofilms from children with early childhood caries (He et al., 2017). In biofilms formed *in vitro*, the presence of *C. albicans* dramatically modifies the physical environment by increasing biomass and EPS production, thereby enhancing biofilm accumulation and stability. Biofilms mixed are considered resistant to conventional therapies and this fact has stimulated the search for alternative antimicrobial agents that can prevent biofilms formation and the onset of associated diseases. Therefore, the objective of this project was to evaluate the effect of the nitrochalcone (NC-E08) on mixed biofilms of *C. albicans* and *S. mutans* formed on hydroxyapatite disks, through cell viability (plating), Microscopy Scanning Electron (MEV), *in vivo* and *in vitro* adhesion and *Galleria mellonella* model infection.

### Results and Discussion

Cell viability results of biofilms formed on hydroxyapatite disks treated with NC-E08, in addition to studies of their *in vivo* toxicity using the *Galleria mellonella* invertebrate model, demonstrated that NC-E08 was able to inhibit planktonic cell growth of *S. mutans* and *C. albicans* with MIC values between 15.75 and 0.97 µg/mL, respectively. For evaluation of the cell viability and SEM, the mixed biofilms were formed on hydroxyapatite disks and were treated with NC-E08 in 3 times per day, during 120h. For the *in vivo* adhesion tests were used Human Gingival Fibroblast Cells (HGF-1-ATCC CRL-2014) and the evaluation of adhesion was made in 3 times (1, 2, and 3 h). Moreover, for the *in vivo* infection was used the *G. mellonella* model, the larvae were infected with pre formed biofilm and treated with NC-E08, Chlorhexidine digluconate and artificial saliva and these were evaluated during 120h.

The results demonstrated that NC-E08 was able to inhibit biofilms of *C. albicans* (25%), *S. mutans* (30%), and the association of these pathogens in approximately 36% for *C. albicans* and 41% for *S. mutans*. In addition, it also showed anti-adhesive capacity in both polystyrene plates (approximately 32% for both) and in human gingival fibroblasts, where NC-E08 showed anti-adhesive capacity at all times studied. In the treatment of infection in the model *G. mellonella*, the NC-E08 presented protection capacity when compared to the larvae that did not receive the treatment.



**Image 1.** Cell viability of biofilms of *C. albicans*, *S. mutans* and mixed of *S. mutans* and *C. albicans* treated with NC-E08(1X MIC), NC-E08 (10X MIC) and digluconate of Clorexidine 0,12%.  $p < 0.05$  – One-way ANOVA with Post Tukey test.

### Conclusions

Nitrochalcone (NC-E08) demonstrated excellent anti-adhesive and anti-biofilm activity on *Candida albicans* and *Streptococcus mutans*, as well as low toxicity *in vivo*, and an efficient activity against the *in vivo* *G. mellonella* model.

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