

**TITLE:** *IN VITRO* AND *IN VIVO* EFFECTS OF IMMUNOGENIC PEPTIDES DERIVED FROM 14-3-3 FROM *P. brasiliensis*

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Paracoccidioidomycosis is a fungal infection restricted to Latin America, caused by dimorphic fungi of the genus *Paracoccidioides*, which is composed by *P. brasiliensis* and *P. lutzii*. Adhesins are the most studied factors of this genus and among them 14-3-3 protein were described by our group. In this study, three peptides (p1, p2 and p3) from the 14-3-3 protein were selected based on *in silico* analysis of immunogenicity and had its effects evaluated *in vitro* and *in vivo* in *Galleria mellonella* and *Caenorhabditis elegans*. For this, the antifungal activity and the cytotoxicity have been evaluated by rezasurin method and by microdilution, respectively. *G. mellonella* was used to evaluate the effect of these peptides in hemocyte concentration and apoptosis protection by flow cytometry. Gene expression of galiomycin and gallerimycin (antimicrobial peptides) was tested by RT-PCR, besides toxicity and efficacy of treatment before the infection with *P. brasiliensis* and *P. lutzii* were evaluated by survival curves. *C. elegans* is a well-characterized model to study immunological response, then, the expression of *abf*, *cnc* and *nlp* gene classes, which are related to the expression of antimicrobial peptides, were evaluated by RT-PCR. None of the peptides showed cytotoxicity in MRC-5 and A549 cells and only p1 exhibited antifungal activity against *Paracoccidioides* spp. When evaluated in *G. mellonella*, the three peptides were not toxic until 200 µg/larva. Moreover, the concentration of hemocytes increased in 1.8 fold for p1 and p2 with 100 µg/larvae. Pre-treatment of *G. mellonella* with 100 µg/larvae of p1, p2 and p3 peptides reduced apoptosis caused by fungal infection. P2 and p3 decreased the apoptosis caused by *P. brasiliensis*, and for *P. lutzii*, p1 and p3 showed the best results. Moreover, the expression of gallerimycin and galiomycin increased 15 and 6.8 folds with p1 and p3 treatment, respectively. Despite of all changes in the *G. mellonella* physiology the increase in the survival of infected larvae in *Paracoccidioides* spp was not observed. Regarding *C. elegans* gene expression, the three peptides were able to increase the expression of the immune response genes. These peptides had important effects on different facets of *Paracoccidioides* spp infection showing important potential for the prevention of infections caused by these fungi.

**Keywords:** *G. mellonella*, *C. elegans*, immunogenic peptides, 14-3-3 protein.

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