

TITLE: PLUMIERIDINE: A NEW MOLECULE WITH ANTIFUNGAL POTENTIAL TO TREAT CRYPTOCOCCAL MENINGITIS

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ABSTRACT:

Cryptococcosis is an invasive fungal infection caused mainly by the pathogenic species *Cryptococcus neoformans* and *Cryptococcus gattii*. This disease affects approximately 1 million individuals annually worldwide with an estimated death rate of 60%. Currently, antifungal drugs show some limitations related to toxicity and resistance, which emphasizes the importance of search for new drugs. Our group identified the antifungal potential of one iridoid - Plumieridine - isolated from the aqueous extract of *Allamanda polyantha* (Apocynaceae) seeds; however, the information about its enzymatic target is still unclear. In this way, the objective of this study is to evaluate the biological activities of plumieridine compound against *C. neoformans*. Plumieridine causes morphological change and decreases polysaccharide capsule in *C. neoformans*. Assays to evaluate the toxicity and cytotoxicity of plumieridine compound were performed using BALB/c mice. Four concentrations (2 mg/kg, 4 mg/kg, 10 mg/kg and 20 mg/kg) of plumieridine were tested in mice, with daily administration for 15 days. These concentrations showed no toxicity for mice. Furthermore, we propose the use of a practical model to monitor the treatment efficacy of these compounds against *C. neoformans*. Our approach involves mice infection with *C. neoformans* fluorescent strain, followed by fluorescence-based imaging analysis of the colonized organs. Mice were infected intranasally with the fluorescent *C. neoformans* strain and treated with plumieridine or fluconazole for 15 days. The lung and brain were excised and examined in an IVIS Lumina instrument to determine fluorescence intensity and for CFU determination. Imaging analysis showed that the treatment with plumieridine reduced the pulmonary and cerebral fungal burden of mice infected with *C. neoformans*. This observation was consistent with the results of CFU determination. Moreover, in order to identify a possible target of plumieridine, studies *in silico* were performed using PharmMapper server. Among the predicted targets, three proteins from the route of pyrimidines were identified, thymidylate synthase, orotidine-5'-phosphate decarboxylase and dihydroorotate dehydrogenase. The structures of thymidylate synthase and orotidine-5'-phosphate decarboxylase were modeled and docked with plumieridine to better understand these interaction modes with *C. neoformans*. The gene expression profile of *C. neoformans* related to route of pyrimidines in the presence of plumieridine is being evaluated by q-RT-PCR. A significant decreased in the transcript levels of thymidylate synthase and orotidine-5'-phosphate decarboxylase genes was detected when cells were cultured in the presence of plumieridine. Our results may contribute for description and development of new antifungal compounds for cryptococcosis treatment.

Keywords: Plumieridine, *C. neoformans*, thymidylate synthase, imaging analysis.

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