TITLE: *In vitro* time-kill study of new antifungal agent against *Paracoccidioides brasiliensis*

AUTHORS: Rodrigues, F. A. V.; Ishida, A. Y.; Faria, D. R.; Sakita, K. M.; Melo, R. C.; Conrado, P. C. V.; Capoci, I.R.; Bonato, F.; Mendonça, P. S. B.; Svidzinski, T. I. E.; Kioshima, E. S.

INSTITUTION: Universidade Estadual de Maringá (UEM) – Paraná – Brasil.

ABSTRACT 2:

Paracoccidioidomycosis (PCM) is a systemic mycosis, restricted to Latin America, and its etiological agent is *Paracoccidiodes brasiliensis* and *Paracoccidioides lutzii*. These therapies have several limitations, such as drug interactions, infusion-related events and nephrotoxicity. The duration of antifungal treatment ranges from months to years and relapses may nevertheless occur despite protracted therapy. Thus, there remains an urgent need for new therapeutic options. The compound studied are selected by virtual screening that have specific target flavoenzyme thioredoxin reductase from *Candida* spp., responsible for the maintenance of the cell redox state. This work aimed at evaluating the action time of a new compound, LMM11, the candidate for new antifungal. The time-kill assay was performed using 24-well plates. The *P. brasiliensis* (Pb18) inoculum was defined as 1×10^4 CFU/mL, counting in neubauer chamber. Four groups were tested, being: control group (yeast + medium RPMI), treatment group with different concentrations of LMM11 - 4, 8 and 16 µg/mL, corresponding to 0.5xMIC, 1xMIC and 2xMIC (data previously found). Cell viability was evaluated by trypan blue assay, at 0, 1, 3, 5, 7 and 14 days of incubation at 35°C, immobile. The time-kill assay showed that from the first day there was a reduction in cell viability 26%. On the fifth day was the highest activity of LMM11, in the three treatment groups with 77, 85 and 92% death, to group 0.5xMIC, MIC, 2xMIC, respectively. At 14 days of treatment the 1xMIC and 2xMIC group killed 91 and 86% of the yeasts of Pb18, respectively. This study showed that the new compound is effectively able to kill *P. brasiliensis* at the concentration of 8 and 16 µg/mL, killing more than 80% of the yeasts of Pb18 *in vitro*.

Keywords: Paracoccidioidomycosis, new molecule, Time-kill.

Development Agency: CAPES, CNPq, Fundação Araucária.