ABSTRACT

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Title of thesis: Employment of alternative animal model, *Galleria mellonella*, for candidate antifungal drugs research

Background: The aim of this thesis was to establish the alternative animal model *Galleria mellonella* for the investigation of novel candidate antifungal agents. Pilot experiments were conducted to optimize key methodological procedures. The main antifungal therapy efficacy by testing treatment outcomes in an animal model with developed fungal infection.

Methods: For the experiments, larvae of *G. mellonella* obtained from in-house breeding were used. Only fully vital larvae were selected for each experiment. The yeast *Candida albicans* strain ATCC 90028 was chosen for infection. The infectious inoculum was administered via injection using a needle through the last left proleg. Larvae were divided into two weight categories for the experiments: 350 ± 25 mg and 450 ± 25 mg. The HISS scoring system was used to assess larval health status. To determine the total fungal burden, larval bodies were homogenized and the resulting material was plated on selective medium, Sabouraud agar. To further quantify the fungal burden, hemolymph was collected from the third segment behind the head and analyzed. The obtained data were subjected to statistical evaluation.

Results: The experiments showed that mortality exceeding the LD_{50} threshold was achieved following administration of an infectious inoculum of *Candida albicans* at a density of 6.48 McF within the observed time frame of 120 hours. Furthermore, it was demonstrated that larvae in the higher weight category, 450 ± 25 mg, exhibited greater resistance to the infectious inoculum compared to those in the lower weight category, 350±25 mg. A noticeable deterioration in larval health due to infection was observed after administration of an inoculum with a density of 5.46 McF. The experiments also revealed substantial variability in the resistance of individual animals to the infectious agent. Additionally, hemolymph was found to be an unsuitable clinical sample for determining fungal burden, as *Candida albicans* is capable of tissue invasion during the course of infection.

Conclusion: In recent decades, the alternative animal model *Galleria mellonella* has gained increasing popularity. This is likely due to its low economic demands, minimal spatial and equipment requirements, and ease of maintenance. Unlike other animal models, ethical regulations do not currently apply to *Galleria mellonella* larvae, making their use less restricted. However, despite being an invertebrate model, it is important to acknowledge that these larvae may still experience pain and stress. For this reason, only a limited number of individuals were included in the pilot experiments conducted in this study. The rise in antifungal resistance and the growing incidence of fungal infections underscore the urgent need for new therapeutic strategies. The pilot experiments contributed to identifying a suitable inoculum density capable of inducing a clearly detectable infection. A high degree of variability in the response to infectious inoculum administration was recorded. For future in vivo evaluation of candidate antifungal compounds, an increased number of test subjects will be necessary. Hemolymph was found to be an unsuitable clinical sample for determining fungal burden.

Keywords: *Galleria mellonella*, antifungal drugs, fungal invasive infections