

# ABSTRACT

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**Title of thesis:** Study of *in vivo* toxicity of selected compounds with the implementation of the invertebrate model *Galleria mellonella*

**Background:** The aim of this diploma thesis was to monitor the influence of the weight of the larvae, or rather its developmental stage, on the results after the administration of selected doses of DMSO. In the experimental work, the toxic impact of DMSO on larvae was evaluated not only from the point of view of mortality. Much attention was also paid to the characteristic features that are commonly observed during tests on *Galleria mellonella* larvae, such as the degree of melanization, the impact on activity and cocoon formation.

**Methods:** For individual experiments, three test groups of six individuals each were created based on weight, one group of larvae weighing  $250 \pm 10$  mg, the second group weighing  $350 \pm 15$  mg and the third group weighing  $450 \pm 15$  mg. Using a Hamilton syringe, a volume of 10  $\mu$ l of the test solution with the appropriate concentration of DMSO was administered to each larva directly into the haemocoel through the last left proleg. The administered doses were: 12.751 g DMSO/kg larval weight; 15.714 g DMSO/kg; 18.850 g DMSO/kg; 22.0 g DMSO/kg larval weight). Two control groups were included. To the first control group has been administered 10  $\mu$ l of pure PBS, and the second group consisted of larvae without any administration. Subsequently, all larvae in the experiments (a total of 108 larvae) were incubated in a thermostat in Petri dishes at a temperature of 37°C. For a period of 168 hours (every time after 24, 48, 96 and 168 hours after administration), the criteria used to determine the "health" score were evaluated for individual larvae as part of the HISS (Health Index Scoring System) evaluation. Death was defined as complete loss of mobility.

**Results:** The larvae in the  $350 \pm 15$  mg weight category showed the highest survival rates, even at the higher DMSO administered doses. The larvae in the  $250 \pm 10$  mg category were less resistant to the  $350 \pm 15$  mg category, but more resistant than larvae in the  $450 \pm 15$  mg category. Apparently, the  $450 \pm 15$  mg group shows the lowest survival rate after exposure to DMSO compared to other weight categories. Intrahaemocoelic administration could be considered analogous to intravenous administration in vertebrates. Both involve the administration of noxa into the body fluid of the animal's circulatory system. However, when comparing all three LD<sub>50</sub> values after 24 hours for *G. mellonella* larvae with acute toxicity values (expressed as LD<sub>50</sub> [g/kg], also after 24 hours) given for three different routes of administration to

selected model animals, it is possible notice a different direction. The LD<sub>50</sub> values of *G. mellonella* larvae after intrahaemocoelic administration were closer to the LD<sub>50</sub> values after peroral administration of DMSO to selected animals.

**Conclusion:** Employment of *G. mellonella* larvae as a model organism brings many advantages. The entire study can be carried out even with a low financial budget. In addition, *G. mellonella* larvae as an animal model are relatively versatile and suitable for many different types of studies. Their innate immunity is in many points of view surprisingly similar to mammal innate immunity. However, *G. mellonella* is at a lower developmental stage, which is why their use in experiments does not require any approval by the ethics committee. The larval weight category of 350 ± 15 mg appeared to be the most resistant, independently of the dose administered. However, the weight category 450 ± 15 mg was the most sensitive. Which supports the employment of larvae of this weight in toxicological screening when it is more beneficial to identify with false positive results than with false negative ones. This approach should enable to study toxicity on other model organisms to identify toxic substances that could in subsequent experiments unnecessarily cause significant suffering or even death to animals. In addition, for preliminary studies it is not desirable to include a lot of animals, the "Three Rs" system of principles must be observed. For toxicological screening, even smaller groups are appropriate, then the aim is to obtain as much data as possible. Therefore, the HISS scoring system was implemented for these experiments, therefore much more information was obtained about the toxic effect of DMSO on the test subjects. The HISS score system has proven to be very useful, and the obtained data could acquire a completely different and more worthwhile way of view. In addition to mortality data, more comprehensive information about the influence of noxa on larval health could be also obtained. The HISS gives us an opportunity to obtain a more rational point of view on toxicity of tested substances in order to further toxicological studies on higher level animals.

**Keywords:** *Galleria mellonella*, *in vivo* toxicity, drug administration, GM Health Index Scoring, dimethyl sulfoxide, The principles of 3 R's, LD<sub>50</sub>, toxicological screening, preclinical trials, reduction