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Uncovering ketamine enantioselective toxicity using *Caenorhabditis elegans* as a discovery platform

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Abstract

Background: The increasing recreational use of ketamine represents a growing public health concern due to the limited understanding of its long-term and enantioselective toxicological effects, as the two ketamine enantiomers are thought to exhibit distinct pharmacological and toxicological profiles [1, 2]. Understanding these differences is essential to better characterize the biological impact of this psychoactive substance. **Objective:** This study aims to investigate the potential enantioselective toxicity of ketamine using *Caenorhabditis elegans* as a discovery platform. **Methods:** Synchronized L1-stage animals of the DC19 [*bus-5(br19)*] strain (~200/condition) were exposed, in liquid medium, to increasing concentrations of ketamine (racemic mixture and (*S*)-ketamine enantiomer; 0 - 10 mM) [3]. Following a 72-h incubation in M9 buffer containing OP50 bacteria as a food source, the animal survival rate was determined by counting the number of live and dead worms after the exposure period. Using sublethal concentrations, we plan to further explore the influence of ketamine on (1) animal development, (2) lifespan, and (3) reproductive behavior. Additional experiments will explore the hatching rate of unexposed F1 embryos laid by exposed animals, as well as the growth of larvae derived from these embryos, to investigate putative heritable toxicological signatures. **Results:** Exposure of synchronized L1-stage animals to ketamine (racemic mixture) at concentrations ≥ 5.0 mM for 72 h significantly decreased survival in a concentration-dependent manner. In contrast, the (*S*)-ketamine enantiomer significantly reduced survival at concentrations ≥ 2.5 mM. Observational evidence indicates that sublethal concentrations of ketamine (both racemic and *S*-ketamine enantiomer) delay animal development, highlighting optimistic perspectives regarding the impact of different ketamine forms on organism-level endpoints. **Conclusions:** Overall, these findings suggest that ketamine induces time-dependent effects in *C. elegans*, with distinct response patterns between the racemic mixture and (*S*)-ketamine, suggesting the presence of enantioselective toxicity. Ongoing analyses will further clarify the impact of these exposures on organismal development, lifespan and reproductive behavior, and potential heritable toxicological signatures, contributing to a deeper understanding of ketamine's toxicological profile.

Keywords: ketamine; enantioselective toxicity; *C. elegans*

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