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Lisdexamfetamine induces concentration-dependent toxicity and developmental effects in *Caenorhabditis elegans*

Mariana Silva-Carvalho[1,2,*], Diana Dias da Silva[1,2,3,4,5], Ricardo Jorge Dinis-Oliveira[1,2,6,7], Daniel José Barbosa[1,2]

[1] Associate Laboratory i4HB-Institute for Health and Bioeconomy, University Institute of Health Sciences - CESPU, Gandra, Portugal
[2] UCIBIO-Applied Molecular Biosciences Unit, Translational Toxicology Research Laboratory, University Institute of Health Sciences (1H-TOXRUN, IUCS-CESPU), Gandra, Portugal
[3] REQUIMTE/LAQV, ESS, Polytechnic of Porto, Porto, Portugal
[4] Associate Laboratory i4HB-Institute for Health and Bioeconomy, Faculty of Pharmacy, University of Porto, Porto, Portugal
[5] UCIBIO-Applied Molecular Biosciences Unit, Laboratory of Toxicology, Faculty of Pharmacy, University of Porto, Porto, Portugal
[6] Department of Public Health and Forensic Sciences and Medical Education, Faculty of Medicine, University of Porto, Porto, Portugal
[7] FOREN-Forensic Science Experts, Lisbon, Portugal

* Correspondence: a29632@alunos.cespu.pt

Abstract

Background: Lisdexamfetamine dimesylate (LDX) is a prodrug of *d*-amphetamine, used for the treatment of attention-deficit/hyperactivity disorder (ADHD) [1, 2]. Despite its clinical use, some knowledge gaps remain regarding its toxicity, including its potential to induce heritable toxicological signatures. **Objective:** To address these gaps, this study aims to use *Caenorhabditis elegans*, a rapid, tractable model with a short lifespan, transparent body, and well-characterized development, as an exploratory discovery platform for efficient toxicological assessment [3]. **Methods:** Synchronized L1-stage animals of the DC19 [*bus-5(br19)*] strain (~200/condition) were exposed, in liquid medium, to increasing concentrations of LDX (0 - 10 mM). Following a 72-h incubation in M9 buffer containing OP50 bacteria as a food source, distinct organism-level phenotypes were assessed. Survival rate was determined by counting the number of live and dead worms after the exposure period. Using sublethal concentrations (0.5, 1.0, and 2.0 mM), we further explored the influence of LDX on (1) animal development, by measuring the body length using Fiji software; lifespan, by monitoring exposed animals every two days throughout their lifespan; and (3) reproductive behavior, by counting the total number of embryos laid by individual F0 exposed animals within a 24-h time window. Further experiments explored 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:** LDX reduced animal survival in a concentration-dependent manner at 3.0-10.0 mM. Concentrations of 0.5-1.0 mM showed no significant effects on F0 development, lifespan, reproduction, or F1 outcomes. At 2.0 mM, directly exposed animals exhibited delayed development, with similar growth retardation observed in F1 progeny at 48-72h post-exposure. Lifespan analysis and reproduction assays at 2.0 mM LDX are currently ongoing. **Conclusions:** LDX demonstrates concentration-dependent toxicity in *C. elegans*. While lower sublethal doses appear well-tolerated, 2.0 mM delays development in exposed animals and in their F1 progeny, warranting further heritable toxicity investigation.

Keywords: lisdexamfetamine dimesylate; heritable toxicological signatures; *C. elegans*

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