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Evaluation of oxidative stress and apoptosis responses in zebrafish (*Danio rerio*) larvae after butylone exposure

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Abstract

Background: The emergence of new psychoactive substances (NPS) in the worldwide drug market raises huge concerns over public health, drug policy and environmental adverse effects [1]. Butylone (BTL) is a synthetic chiral cathinone [2] reported in wastewater effluents and despite its increasing presence in freshwater ecosystems, no studies were found on the BTL toxic evaluation in fish, including the well-known zebrafish (*Danio rerio*) model. Zebrafish is widely used to investigate the potential adverse effects of different toxics, including NPS, specifically during sensitive early-life stages [3]. **Objective:** The main goal of this study was to evaluate the potential toxic effects of BTL on the apoptosis response, enzymatic and non-enzymatic biomarkers levels. **Methods:** Zebrafish embryos with ≈ 3 hours post-fertilization (hpf) were exposed for 96 hours to different concentrations of (*R,S*)-BTL (0.01, 0.1, 1, 10 and 100 µg/L). After exposure, the determination of apoptosis level was carried out by measuring the fluorescence (excitation/emission 535/590 nm) of 10 larvae from each repetition/concentration, immersed in acridine orange dye. Furthermore, 30 larvae from each treatment concentration were collected to assess the effects on reactive oxygen species generation, enzymatic activities (superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, lactate dehydrogenase, acetylcholinesterase, and glutathione S-transferases) and non-enzymatic biomarkers (protein carbonyls, thiobarbituric acid reactive substances, reduced glutathione, and glutathione disulphide). **Results:** No statistically significant differences were observed in any of the different biomarkers analysed. **Conclusions:** These findings suggest that the exposure to environmentally relevant concentrations of BTL (0.1 µg/L) during the early life stages of zebrafish may not alter the redox homeostasis with the induction of oxidative stress nor induce apoptosis, reflecting no adverse effects for acute exposure. However, further research is needed to investigate in more detail the impacts of BTL on other biomarkers, like genotoxicity, behavioural changes, and focus on chronic exposure and multigenerational effects, consequently improving the accuracy of environmental risk assessment.

Keywords: psychoactive substances; butylone; biochemical biomarkers; apoptosis; *Danio rerio*

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