

ENDOCRINE EFFECTS OF AZITHROMYCIN, NIMESULIDE AND THEIR  
ASSOCIATION IN ADULT ZEBRAFISH.

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**INTRODUCTION:** The increasing use of antibiotics and anti-inflammatory drugs is a global concern due to bacterial resistance and environmental toxicity. Azithromycin (AZI) and nimesulide (NIME) are commonly associated in treatment protocols and often used without a prescription. Additionally, improper drug disposal in the environment causes contamination. These substances are found still active and negatively affect non-target organisms, for example, causing changes in fish's the neuroendocrine axis, such as the hypothalamic-pituitary-interrenal axis, which has cortisol as its final product, the most consolidated marker of stress responses. **OBJECTIVES:** To evaluate whether exposure to AZT, NIME and their association induces an endocrine stress response (cortisol) in adult zebrafish (*Danio rerio*). **MATERIALS AND METHODS:** The zebrafish population consisted of 60 fish (1:1 female/male), housed in glass aquariums (4 L, density 1 fish/L), and acclimated for seven days, according to the species's maintenance conditions. Subsequently, they were distributed into five groups (control, DMSO, AZT, NIME and AZT+NIME) with 12 fish each. The concentration used was 12.5 µg/mL for AZT and 0.4 µg/mL for NIME. The 15-min exposure was conducted once a day for four consecutive days and, after exposure, the animals were returned to the original aquarium. On the 5th day, the fish were captured, euthanized and stored for extraction of body cortisol and measurement with the enzyme-linked immunosorbent assay (ELISA) kit. Since the

DMSO group did not differ from the control, data from this group were excluded from the analysis (two-way ANOVA, followed by Tukey). RESULTS AND CONCLUSION: The presence of NIME, AZT and their association significantly increased the body cortisol concentration in exposed fish. Elevated cortisol levels negatively influence the growth, reproduction, metabolic and immune systems of animals, making them susceptible to diseases and predation. Thus, survival and population development can be harmed by the presence of pharmaceuticals in the water. Our study emphasizes the need for awareness about the use of these substances and stricter regulations regarding their disposal.

KEYWORDS: Environment contamination; drugs; cortisol;

FUNDING: National Council for Scientific and Technological Development (CNPq): Universal Call 2023 (403048/2023-0) and Research Productivity Grant for LJGB (302167/2022-6).