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Cardiac and Cerebellar Histomorphology and Inositol 1,4,5-Trisphosphate (IP_3R) Perturbations in Adult *Xenopus laevis* Following Atrazine Exposure

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Abstract: Despite several reports on the endocrine-disrupting ability of atrazine in amphibian models, few studies have investigated atrazine toxicity in the heart and cerebellum. This study investigated the effect of atrazine on the unique Ca^{2+} channel-dependent receptor (Inositol 1,4,5-trisphosphate; IP_3R) in the heart and the cerebellum of adult male *Xenopus laevis* and documented the associated histomorphology changes implicated in cardiac and cerebellar function. Sixty adult male African clawed frogs (*Xenopus laevis*) were exposed to atrazine (0 µg/L (control), 0.01 µg/L, 200 µg/L, and 500 µg/L) for 90 days. Thereafter, heart and cerebellar sections were processed with routine histological stains (heart) or Cresyl violet (brain), and IP_3R histochemical localization was carried out on both organs. The histomorphology measurements revealed a significant decrease in the mean percentage area fraction of atrial (0.01 µg/L and 200 µg/L) and ventricular myocytes (200 µg/L) with an increased area fraction of interstitial space, while a significant decrease in Purkinje cells was observed in all atrazine groups ($p < 0.008$, 0.001, and 0.0001). Cardiac IP_3R was successfully localized, and its mean expression was significantly increased (atrium) or decreased (cerebellum) in all atrazine-exposed groups, suggesting that atrazine may adversely impair cerebellar plasticity and optimal functioning of the heart due to possible disturbances of calcium release, and may also induce several associated cardiac and neural pathophysiologies in all atrazine concentrations, especially at 500 µg/L.

Keywords: atrazine; Purkinje; cerebellum; myocytes; toxicology; IP_3Rs

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1. Introduction

Atrazine is designed specifically to impair the physiology of weeds [1]; however, the physical and chemical properties of atrazine, such as its half-life, slow degradation [2], and solubility in different mediums, contribute to its persistence in the environment and, consequently, its contamination of ground, surface, and drinking water [3–5]. This suggests a constant presence of atrazine and its degradation products in the environment and, therefore, in drinking water and aquatic and terrestrial environments [3], with inevitable pathological consequences.

The effects of atrazine exposure have been widely reported such as impaired development in fish [6] and perturbed immunology and growth in *Xenopus laevis* [7,8]. However, most information on atrazine-induced effects in adult animal models have focused mostly on its endocrine-disrupting abilities, particularly in *Xenopus laevis* gonadal morphology [9–11]. Furthermore, atrazine and its metabolites can bio-accumulate in aquatic microbiota with adverse aquatic ecosystem implications [12] and can induce several organ

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study, IP_3Rs were successfully localized in the atrium and ventricles of frog hearts, and the results showed a significant increase in atrial IP_3Rs and a non-significant increase in ventricular IP_3Rs following atrazine treatment, which may be related to the differences in function of atrial and ventricular myocytes [43,44]. Additionally, elevated IP_3R expression is reputably observed in cardiac ventricular hypertrophy, heart failure, atrial fibrillation, and dilated cardiomyopathy [45,46], which mirrors our histomorphometry observations. These elevated cardiac IP_3R levels are also suggested to compensate for waning RYRs in human heart diseases [45,47].

The observed micromorphological changes in the heart and cerebellum following atrazine treatment and IP_3R perturbations in both organs (peripheral and central toxicity) indicates atrazine's potential to disrupt cardiac and cerebellar excitability with attendant dysfunctions. These histopathological observations together suggest impaired motor agility, mild ventricular cardiomyopathy, and progressed atrial arrythmias, all of which severely impair the ability of *Xenopus* to thrive in its environment. The results further emphasize that caution should be applied to atrazine use and application and regulatory guidelines should be enforced, as significant organ toxicities are evident at the environmentally relevant concentration (0.01 µg/L), which may have grave consequences in aquatic animal life conservation, the ecosystem, and general environmental health.

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Data Availability Statement: The supporting data reported in this study will be made available publicly during the course of publication, if this manuscript is accepted.

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