



International Journal of Chemistry and Pharmaceutical Sciences

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Research Article

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Assessment of Genotoxic Effect of Atrazine on *Poecilia Sphenops* Using Micronucleus Assay

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ABSTRACT

Atrazine is one of the most effective and cheapest herbicides in the world and is consequently used more frequently than any other herbicide. Atrazine is frequently detected in waters, and has been known to affect reproduction of aquatic flora and fauna, which in turn impacts on the community structure as a whole. In the present study we aimed to assess the genotoxic effects of atrazine on *Poecilia sphenops* using the micronucleus test in peripheral blood erythrocytes. Fish were exposed to atrazine (2.5, 1.25, 0.83 µg/L) and control also maintained simultaneously for 30 days. Our outcome revealed significant increases in the frequencies of micronuclei in erythrocytes of *Poecilia sphenops*, subsequent exposure to atrazine and thus demonstrated the genotoxic potential of this herbicides on fish.

Keywords: Micronuclei, Atrazine, *Poecilia sphenops*, erythrocytes

ARTICLE INFO

CONTENTS

1. Introduction	293
2. Experimental	294
3. Results and Discussion	294
4. References	295

Article History: Received 05 March 2016, Accepted 29 April 2016, Available Online 27 June 2016

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Manuscript ID: IJCPs2937



PAPER-QR CODE

Citation: S. Vasanth. Assessment of Genotoxic Effect of Atrazine on *Poecilia Sphenops* Using Micronucleus Assay. *Int. J. Chem, Pharm, Sci.*, 2016, 4(6): 293-295.

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1. Introduction

Chemical contaminants are major concern in a polluted environment include persistent organic pesticides, radionuclides, oils, nutrients, heavy metals, etc. Some of these chemicals are known genotoxicants and can cause damage to the genetic material of the exposed organism International Journal of Chemistry and Pharmaceutical Sciences

(Shugart, 1990b). Apart from the enzymatic biomarkers, other parameters, such as DNA damage, should be evaluated for assessing the consequences of exposure of herbicides (Mitchelmore and Chipman, 1998). Damaged DNA may potentiate subsequent deleterious cellular events

such as disease (e.g., cancer) (Phillips, 1983) and reduced reproductive competence (Zenzen, 2000).

There is a growing concern over the widespread use of herbicides that contaminate the aquatic environment. As a consequence of agriculture runoff, rains, irrigation waters, wetland applications, etc., herbicides are released into aquatic ecosystems. Among the various herbicides used, atrazine (2-chloro-4-ethylamino-6-isopropylamino-1, 3, 5-triazine), because of its high stability and persistence in the aquatic environment attracts special attention. The purpose of this study was to investigate the effects of atrazine herbicide on micronucleus formation in erythrocytes of *Poecilia sphenops*.

2. Experimental

2.1 Test Species: *Poecilia sphenops*

In the present study, viviparous *Poecilia sphenops* are used as experimental animal, to understand the mechanism and ability of toxicity in response to the herbicides. *Poecilia sphenops* is a viviparous fish popularly known as black molly. The shape of black molly is somewhat similar to that of sword tail. But here the sword like elongation of caudal fin is absent and dorsal fin is larger in size. The whole body is black in colour. It grows up to 6 to 10 cm.

2.2 Experimental procedure

Mature fishes were obtained from local ornamental fish dealer and maintained in the aquarium separately and intended for breeding within a week time. The laboratory temperature, $27 \pm 0.5^\circ\text{C}$ and normal illumination (approx 12h light and 12h dark) with the ration of pieces of earthworm twice a day were ideal. The following physicochemical characteristics: temperature $27 \pm 0.5^\circ\text{C}$, pH 6.3 ± 0.4 , Dissolved oxygen 6.5 ± 0.4 mg/L, salinity 0.6 ± 0.03 ppt, nitrite 0.04 ± 0.008 mg/L, and hardness (as CaCO_3), 19.2 ± 0.05 mg/L. These parameters were measured according to the experimental procedures described in Standard Methods for the Examination of Water and Wastewater (APHA, 1998). Twenty fishes in each group were exposed to three different concentrations ($1/10^{\text{th}}$ of LC 50, 2.5ppm, $1/20^{\text{th}}$ of LC 50, 1.25ppm and $1/30^{\text{th}}$ of LC 50, 0.83ppm) of atrazine and control also maintained simultaneously.

2.3. Sample Preparation for Micronucleus Test

At the end of 30 days of atrazine exposure, Peripheral blood samples were obtained from the caudal vein of the *Poecilia sphenops* and smeared on clean slides. After fixation in pure ethanol for 20 min, slides were left to air-dry and then the smears were stained with 10% Giemsa solution for 25 min. Observations were made using an Olympus research microscope. Five slides were prepared from each group of fish. 1000 erythrocytes were scored from each slide under 1000- fold magnification to determine the frequency of notched nuclei, lobed nuclei, budding, fragmenting and also micro nucleated cells, which was calculated as per 1000 cells (%). NAs were classified according to Carrasco *et al.*

3. Results and Discussion

The frequencies of micronuclei nuclear abnormalities are observed in erythrocytes of *Poecilia sphenops* exposed to International Journal of Chemistry and Pharmaceutical Sciences

atrazine. The frequencies of micronuclei, observed in erythrocytes of *Poecilia sphenops*, atrazine to the tested three sub lethal concentrations of the Atrazine herbicide showed differences among themselves. The kinds of the nuclear alterations (Fig.1) most frequently observed in erythrocytes of *Poecilia sphenops* exposed to the Atrazine are binucleated cells, cells with “blebbed” nuclei, cells with “lobed” nuclei.

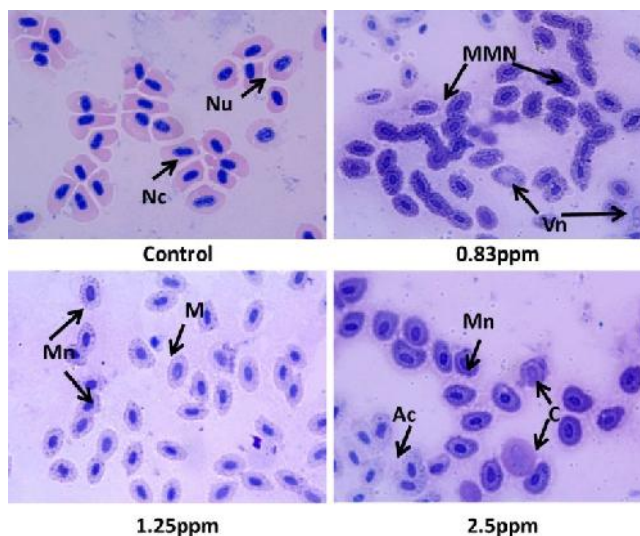


Figure 1: Atrazine Induced Micronucleus and Nuclear Alterations in Erythrocytes of *Poecilia sphenops*

Nu-Nucleus, Nc-Normal cell,
MMN –Multiple Micronucleus,
Vn- Vacuole nuclei,
Mn-Micronucleus, M-Microcyte,
C-Cariolysis, Ac-Altered cell.

Discussion

Molecular markers of the biological effects of contaminants on organisms (Biomarkers) can be used as diagnostic and prognostic early warning tests to detect and assess the effects of pollution, particularly low concentrations of complex mixtures of contaminants, on environmental quality (Livingstone, 1993). In the present study, genotoxicity are evaluated through micronuclei assay, for atrazine impact in *Poecilia sphenops*. Present results point out that the Nuclear Alterations in Erythrocytes of *Poecilia sphenops* increases with increasing atrazine concentration, indicating that the levels of micronuclei formation imposed by atrazine could be quantified by micronuclei assay. Araldi, *et al.* (2015) reported that using the micronucleus assays for genotoxicity studies. The micronucleus test in peripheral erythrocytes provides a possible approach to monitor the effects of environmental genotoxic agents in fish. The micronucleus assay in erythrocytes of fish was widely used as a marker for environment pollutant (Gustavino *et al.*, 2001). The pesticide cause significant damage in the red blood cells of *Channa punctatus* even at a lower concentration (Sawhney and Johal 2000). The study by Kan *et al.* (2012) suggested deltamethrin to be the causative agents for creating micronucleus gills of *Oreochromis niloticus*. The same results were observed in

studies with the Atrazine-Based Herbicide on Fish *Carassius auratus*. (Cavas, T. 2011). The finding of the present study demonstrates that Atrazine herbicide have micronuclei nuclear abnormalities in *Poecilia sphenops*. The micronucleus test as genotoxicity endpoints and reported significant increases in the frequencies of micronuclei formation exposure to atrazine. These findings may provide evidence for atrazine induced genotoxicity that could be useful for investigating the effects of toxic blooms on wild fish populations.

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