Research Article

Histoarchitectural Alterations in Various Tissues of *Poecilia sphenops* Exposed Three Sub Lethal Concentrations of Atrazine

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ABSTRACT

Atrazine can affect normal physiological processes of many aquatic organisms, especially fish. In the current study, the *Poecilia sphenops* were exposed to 3 sub lethal concentrations 0.83ppm, 1.25ppm and 2.5ppm of atrazine and control also maintained simultaneously. Most histological changes were noticed in the group exposed to 1.25ppm and 2.5ppm. Hepatocytes show changes like wrinkled blood vessel, necrotic area, disintegrating acinar cell, cell debris, perivascular necrosis, vascular damage with swelling, Fragmented ova with abnormal shape and large number of early vitellogenic oocytes were observed in ovaries, the structural modifications in gill are edema, epithelial lifting, and thickening of the primary lamellar epithelium and fusion of secondary lamellae

Key words: Atrazine, *Poecilia sphenops*, Tissues, Histoarchitectural, Sub Lethal Concentrations.

INTRODUCTION

Herbicide contamination of surface waters derived from agricultural industries is a problem of worldwide importance due to aquatic contamination by these products.[1] Herbicides are the most widely used toxic chemicals for various purposes in gardens, horticulture and agriculture. Water often serves as the sink for these chemicals after their application in the different fields. Developing countries have encounter the maximum adverse effects of these chemical compounds in ecosystem on fish production.[2] Besides the change of available plankton food, the application of herbicides has hazarduous effects on the fish.[3] The herbicide may produce an immense disruption of the ecological balance causing damage to non-target organisms including fish.[4,5]

Atrazine is relatively persistent in freshwater, with a half-life between 8 and 350 days, depending on the physio-chemical.[6,7]

The extensive use of atrazine has made this compound a focus for environmental impact studies.[8]

Depending upon the dose life stage and the species, studies on the toxic effects of atrazine in fish have indicated a great variability in the responses.[9]

Histological alterations can be used as direct indicators for the effects of various anthropogenic pollutants on organisms and reflect the overall health of the entire population in the ecosystem. These histological biomarkers are closely related to other biomarkers of stress since many pollutants have to undergo metabolic activation in order to be able to provoke cellular change in the affected organism.

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MATERIALS AND METHODS

Test Species: *Poecilia sphenops*

The newly hatched juveniles/fries were separated from their respective mother and maintained in 100 L tank. A total of 200 fry (0 day old fry) were separated and used in four equal treatment groups. Fifty individuals' fries in each group were exposed to three different concentrations ($1/10^{th}$ of LC_{50}, 2.5ppm, $1/20^{th}$ of LC_{50}, 1.25ppm and$1/30^{th}$ of LC_{50}, 0.83ppm) of atrazine and control also maintained simultaneously.

Histopathological analysis

At the end of 100 days, the *Poecilia sphenops* from each test groups as well as control group were sacrificed gills, liver and ovary were dissected out and fixed in 10% formalin. Macroscopic characteristics for classifying gonadal maturity correspond to were made. [10,11] Female characteristics included the presence of eggs visible to the naked eye as light yellow to reddish appearance from increased vascularisation of the ovary. The characteristics of mature males included white in appearance and relative enlargement of testes within the body cavity.

RESULTS

The histology of gills, liver and ovary of *Poecilia sphenops* are briefly described. The structures of liver, gonad and gill were dealt for all tested groups.

Histopathological structures are observed in the liver of control fish. The structural details of male and female *Poecilia sphenops* liver are shown in Figure 1 and 2. In the fish exposed to atrazine the hepatocytes show changes like wrinkled blood vessel, necrotic area, disintegrating acinar cell, cell debris, perivascular necrosis, vascular damage with swelling. The livers of fish exposed to atrazine show several histopathological changes, and the frequency of these changes increase with increasing concentration.

Ovaries are assessed for the presence and general development of oogonia and oocytes. The ovary of females contains all the developmental stages of oocytes, a small number of yolkless oocytes, various sized vitellogenic oocytes, maturing or fully matured oocytes and ovulated eggs in the ovarian cavity. Atrazine causes significant increase in ovarian follicular size compared to control (Figure 3). Fragmented ova with abnormal shape and large number of early vitellogenic oocytes and traces of reovulatory degenerated oocytes are observed in the experimental fish while normal arrangement of ova is found in case of control fish. Therefore, the atrazine exposed *Poecilia sphenops* ovary

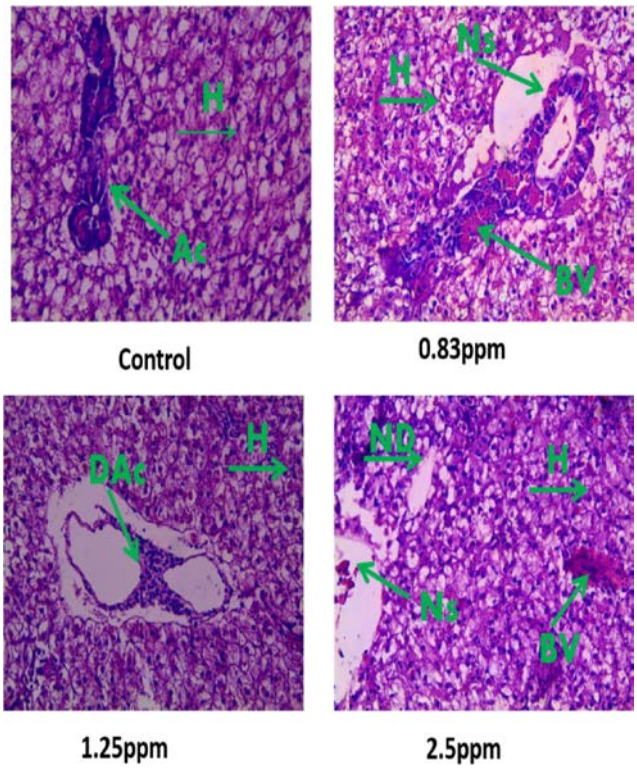

Figure 1: Impact of Atrazine on Liver of Male *Poecilia sphenops*. He-Hepatocytes, Ac- Acinar cells, Nd- Necrotic debris, BC- Blood vessel, Ns-Necrotic spot, DAc- Degenerating Acinar cells. Hematoxylin-Eosin, 40x.

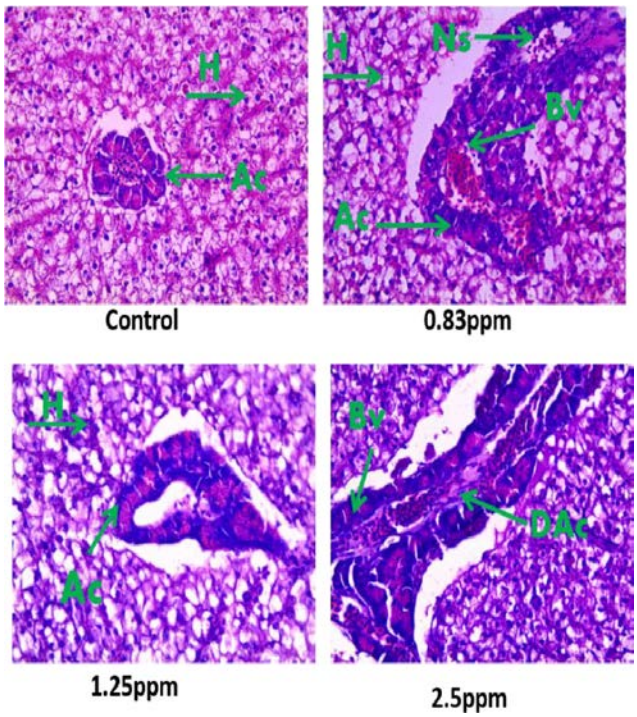

Figure 2: Impact of Atrazine on Liver of Female *Poecilia sphenops*. H-Hepatocytes, Ac-Acinar cell, BV- Blood vessel, Ns-Necrotic spot, DAc- Degenerating Acinar cells. Hematoxylin-Eosin, 40x.

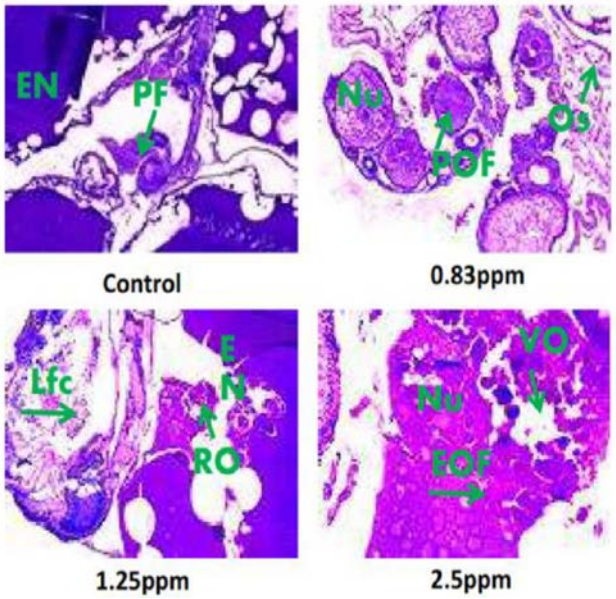

Figure 3: Impact of Atrazine on Ovary of *Poecilia sphenops.* Nu- Nucleus, PF- Primary Follicle, EN- Enlarged Nucleus, VO- Vitellogenic oocytes, POF-Post Ovulatory Follicles, Os- Oocytes shrinkage, EOF- Early Ovulatory Follicles, Lfc- Loss of follicular cells, RO- Reduced Oocytes. Hematoxylin-Eosin, 40x.

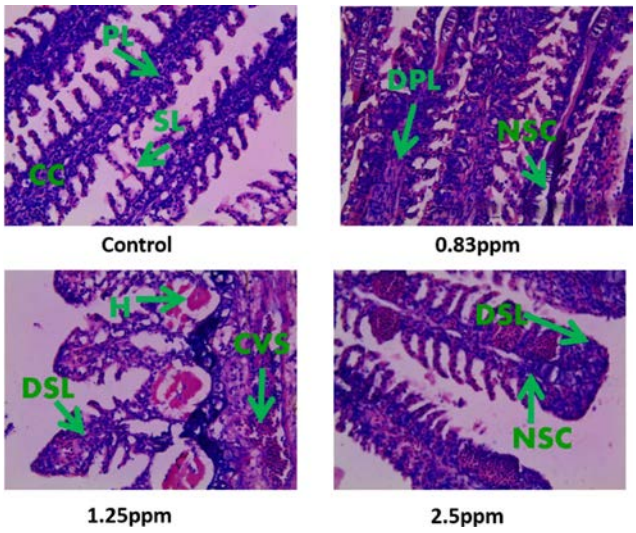

Figure 4: Impact of Atrazine on Gill of Male *Poecilia sphenops.* PL– Primary Lamella, SL-Secondary Lamellae, CC- Chloride Cells, CVS-Central venous sinus, DSL-Disintegrating Secondary Lamellae, NSC-Naked Supporting Cartilage. Hematoxylin-Eosin, 40x.

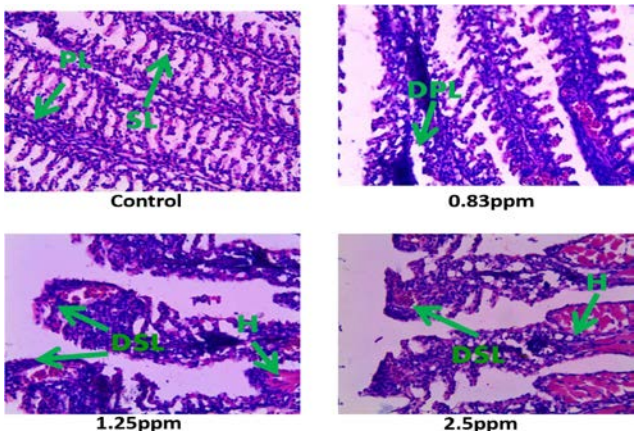

Figure 5: Impact of Atrazine on Gill of Female *Poecilia sphenops.* SL-Secondary Lamella, PL–Primary Lamella, DSL-Disintegrating Secondary Lamellae, H-Hemorrhage. Hematoxylin-Eosin, 40x.

oocytes contain shrinkage of nuclei, irregular oocyte maturation, ruptured ovarian follicles and follicular cells loss.

Normal histopathological morphology was observed in the gill of the control fish. The structural details of the gill of control *Poecilia sphenops* is shown in Figure (4 and 5).

Atrazine exposed fish gill shows severe aneurism in the secondary lamellae. Histopathological changes which may contribute to the problems related to respiration resulted in hypoxia and respiratory failure. In addition, the fish showed problems related to ionic, acid and base balance. Ruptures of the gill epithelium observed are direct responses to the action of atrazine. In the present study the major changes in gills are edema, epithelial lifting, and thickening of the primary lamellar epithelium and fusion of secondary lamellae.

DISCUSSION

Histopathological results indicated that gill is the primary target tissue affected by atrazine, the effects of atrazine on the major osmoregulatory organs, gill of *Poecilia sphenops* in this study. Significant changes in gill like increase of epidermal thickness, fusion of secondary lamellae, hyperplasia, club-shaped cartilaginous tissue, aneurysm, and necrosis in epithelium region are found in *Poecilia sphenops* exposed to atrazine. The lesions became more severe with the increase of the concentration of atrazine. All these lesions may impair respiratory functions in the gill of *Poecilia sphenops*. Fish fails to get adequate oxygen for total metabolic activities. Increased thickness of the epithelial layers has been reported in pesticide exposure.[12]

These results suggested that exposure to atrazine at higher dose could leads to histopathological damages on gill of *Poecilia sphenops*. Such lesions also been observed in gill of rainbow trout and common carp (*Cyprinus carpio*).[13,14]

Histopathological effects of sublethal concentrations of monocrotophos on the gills of *Anabas testudineus* was reported.[15] De Silva and Samayawardhena also observed the effect of chlorpyrifos in guppy *Poecilia reticulata*.[16] As a result of effect of chlorpyrifos the histological lesions are Shorter gill lamellae, fusion, complete destruction of lamella, increased vacuolation, irregular appearance of gill lamellae. The histopathological changes in gill of freshwater fish *Gnathonemus ptersii* exposed to pollutants can result in hypoxia, respiratory failure problems with ionic and acid-base balance.[17] Damages incurred to the gills of fish treated with paraquat might be due to oxidative damages to the cells of this tissue.

The liver is the primary organ for metabolism, detoxification of xenobiotics and excretion of harmful substances.[18] Liver has the ability to detoxify pesticides but elevated concentrations of these compounds result in structural damage.[19] In present study histological lesions in the liver shows cloudy swelling of hepatocytes, hepatocytes with some pycnotic nuclei, lipoid vacuoles and focal necrosis. In another study, *Labeo rohita* exposed to cypermethrin, the following leisons like hyperplasia, and disintegration of hepatic mass and focal necrosis.[20]

Crestani *et al*., also observed alterations in the liver after pesticide exposed to silver catfish (*Rhamdia quelen*).[21] The observed histological changes in liver are more evident in fish exposed to higher dose of atrazine. Similar histopathological effects are observed in liver of *Poecilia sphenops* due to ATZ.[22]

The hepatotoxic effect of ATZ inhibits the activity of key enzymes of glyconeogenesis such as hexokinase, glycogen synthase, and glucokinase and it can explain decrease in body weight of animals observed in our study.[23] This finding agrees with Vasanth *et al*., who studied fish exposed to low doses of ATZ (2 mg/kg) and observed the decrease of glycogen and the increase of lipids in the liver.[22]

The ovaries in matured female *Poecilia sphenops* contain oocyte at various developmental stages concomitantly. The increased level of atretic oocytes in the ovaries of *Poecilia sphenops* is observed. Atrazine treatment shows increased size of matured follicles compared to the controls. Reduction in size of matured oocytes along with vacuolation of cytoplasm, complete loss of normal configuration of ovary, necrosis, elongated ovarian follicles, and fragmented ova with abnormal shape are observed. In present study atrazine affects normal structure of ovary and shows many structural and degenerative changes as above in *Poecilia sphenops*. Similar result was observed in the ovaries of a fresh water teleost *Channa striatus* induced by sub lethal doses of pesticides.[24] In *Poecilia latipinna* affected by *Tribulus terrestris* shrinkage of oocytes nuclei, irregular oocyte maturation, absence of nucleus, rupture of ovarian follicle, and loss of follicular cells were observed.[25,26] The nucleoli were either reduced in size or in number or absent or necrotic surrounded by vacuole.

CONCLUSION

The histological observation indicates that exposure to atrazine causes destructive effect in the gill, liver and ovary of *Poecilia sphenops*. Such histological alterations observed in this study could result in severe physiological problems.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

ATZ: Atrazine; **LC:** Lethal Concentrations; **PPM:** Parts Per Million.

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