Histopathological Changes in the Gill of *Labeo rohita* (Hamilton) Fingerlings Exposed to Atrazine

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**Abstract:** Atrazine (2-chloro-4-ethylamino-6-isopropylamino 1,3,5-triazine) is a widely used selective herbicide. Due to its persistence, it is present in the surface waters, contaminating non-target organisms such as fish. Herbicide atrazine was administered to *Labeo rohita* fingerlings in 120 hours. The used dose of atrazine was 0.18 mg/l for 120 hours (LC$_{50}$). The histopathological changes in the gill tissue like epithelial hyperplasia, curling of secondary lamellae and changes in chloride cells, besides these changes pyknotic nuclei, vacuolization, degradation of epithelial cells and pillar cells, were noticed.

**Key words:** Histopathological changes • *Labeo rohita* • Gill • Atrazine

**INTRODUCTION**

Atrazine (2-chloro-4-ethylamino-6-isopropylamino 1, 3, 5-triazine) is a white crystalline solid synthetic herbicide that is used as to kill broad leaf weeds in agricultural and roadway applications [1, 2]. In freshwater invertebrates atrazine has been found to affect hydromineral balance and gill function in crabs[3]. It can be detected in nearly every surface water system in the world. Fish are very susceptible to bioaccumulation in their fatty tissues, as they take up lindane residues from the water through the gills and skin [4]. The exposure to chemical contaminants can induce a number of lesions and injuries to different fish organs suitable for histopathological examination in searching for damages to tissue and cells [5, 6]. Although damages have been observed in the gill arches, liver, kidney, olfactory epithelium and nervous system[6].

In fish, gill are vital organs for their respiratory and osmoregulatory functions. Respiratory distress is one of the early symptoms of pesticide intoxication [4]. Considerable interest has been shown in recent years in histopathological study while conducting sub-lethal tests in fish. Tissue changes in test organisms exposed to a sub-lethal concentration of toxicant are a functional response of organisms which provides information on the nature of the toxicant. Histological changes associated with pesticides in fish have been studied by many authors [7-9]. In the present investigation was an attempt to observe the histological alterations in the gill of *Labeo rohita* exposed to atrazine.

**MATERIALS AND METHODS**

Healthy *Labeo rohita* fingerlings were procured from the freshwater form located in Puthur, Nagappattinam district. They were acclimatized for a maximum period of 15 days in the laboratory condition. The fingerlings each measuring 4.5 to 6.0 cm in length 5-6 g in weight are used. They were exposed to sublethal concentration of atrazine 0.18 mg/l for a period of 120 hrs. The gill pathology was examined after 120 hrs. An equal number of control fish were examined at similar intervals for histological comparison. The gill was removed and fixed in Bourin’s fluid and then processed for microtome sectioning at 8µm and stained with haematoxylin, eosin and mounted in DPX.

**RESULTS AND DISCUSSION**

The gill is made up of filaments of primary lamellae arranged in double rows. Secondary lamellae arise from these filaments. The secondary lamellae are lined by a squamous epithelium. Histopathological changes in the gill of fishes due to pesticides and other contaminants have been reported earlier[10].

In the present study the observed changes are excessive mucus secretion, lifting up of the epithelium and lamellar fusion. The lifting of the epithelium increases the distance through which the toxicant has to travel to reach the blood stream (Fig. B, C). No recognizable changes were observed in the gills of the control fish. Each gill
consisted of a primary filament and secondary lamellae (Fig. A). The primary lamellar epithelium was one or two cell layers thick. Chloride cells were visible along the primary lamellar epithelium especially at the bases of secondary lamellae. Since the gills are the primary route for the entry of pesticide. According to Leino et al. [11], the gill of pearl dace and fathead minnows from environmentally polluted Canadian lakes exhibited various cellular, histological and histopathological changes, which may contribute to problems related to respiration and acid-base balances. The severe damage in rupture of the gill epithelium resulted in hypoxia and respiratory failure. In addition, the fish showed problems in relation to ionic acid and base balance. Ruptures of the gill epithelium observed are direct responses to the action of atrazine. Roy and Datta, [12] reported that slight hyperplasia of gill epithelium in *Pineaus monodon* exposed to gusathion, a commonly used organophosphate. They further reported that inflammatory alterations of lamellar epithelium and hyperplasia were reported in the gills of freshwater major carp *Cirrhinus mrigala* (Hamilton) during 48 hrs exposure to sublethal dose of malathion.

In the present investigation freshwater fish *Labeo rohita* (Hamilton) exposed to sublethal concentrations of atrazine to hyperplasia of gill filaments, fusion of gill filaments due to separation, necrosis of gill epithelium, degeneration of pillar cells and the development of vacuoles in the epithelium are the prominent pathological changes (Fig. D and, E). The damage of gill of fish exposed to the sublethal concentrations were severe. Shortend and clubbing of

Fig. 1: A. Gills from control fish showing primary lamellae and secondary lamellae arising from these, parallel with them and perpendicular to the filament arcis (H and E × 400). B and C. Shortening of secondary lamellae and edematous separation of the epithelial layer (--) with necrotic cells occupying in the interlamellar space (H and E × 400). D and E. Swelling of the epithelium and fusion of secondary lamellae, hyperplasia, epithelial living in the base and tips of secondary lamellae (H and E × 400).
ends of the secondary gill lamellae, fusion of adjacent secondary gill lamellae and necrosis in the primary lamellae were well marked. Hyperplasia and hypertrophy of nuclei were also seen. Besides, there were changes in pyknotic nuclei, vacuolization and degeneration of epithelial cells and pillar cells, and lifting of the epithelial layer from the secondary lamellae were also significant (Fig. D and E).

Similar changes were observed in freshwater fish exposed to sublethal concentrations of monocrotophos to fenvalerate in *Labeo rohita* by Tilak et al. [13] and to cypermethrin in *Labeo rohita* Veeraiah, [14].

In fish exposed to 0.18 mg/l atrazine concentration (120 hours LC50) in the present study, the major changes in gills were edema, epithelial lifting, thickening of the primary lamellar epithelium and fusion of secondary lamellae. (Fig. D and E). Histopathological changes of gills such as hyperplasia and hypertrophy, epithelial lifting, aneurysm and increase in mucus secretion have been reported after the exposure of fish to a variety of noxious agents in the water, such as pesticides and phenols Nowak [15]. All these lesions may impair respiratory function. Filament cell proliferation and lamellar pavement cell hypertrophy reduce the inter lamellar space and may cause a complete lamellar fusion reducing the total surface area for gas exchange Nowak [15]. Otherwise, they increase in the distance of the water-blood barrier, which together with epithelial lifting and the increase in mucus secretion may drastically reduce the O2 uptake and if the damping agent is not removed, can lead to the rupture of blood vessels with small haemorrhage focus.

In the present study, histological changes have been related to atrazine concentration. It can be concluded that gill alterations as a result of herbicide exposition of fish may serve as a sensitive biomarker for the toxicity of sublethal concentrations of herbicides as well as other pollutants. However, complementary studies are necessary for a better understanding of its deleterious effects.

**REFERENCES**

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