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Research Article

HISTOPATHOLOGICAL CHANGES IN THE GILL AND LIVER OF FRESHWATER FISH *LABEO ROHITA* (HAMILTON) EXPOSED TO NOVALURON

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ABSTRACT

. Gill and liver tissues of Freshwater fish *Labeo rohita* were examined after exposure to sublethal concentrations of novaluron for 15days. Lesions were observed in gill and liver, lamellar fusion, hyperplasia epithelial lifting formation of vacuoles, degenerated hepatic tissues, necrosis and hypertrophy were observed on the toxicant treated fish. None of these lesions were observed in control fish.

Keywords: Lesions, vacuoles, gill and necrosis

INTRODUCTION

Aquatic ecosystem is the final sink for the many chemicals used in industry and agriculture has a global problem, the continuous release of these chemicals impair water quality and become unsuitable for aquatic organisms due to their persistence, bioaccumulation, toxicity and biomagnification in food chain and ecological balance (Palaniappan *et al*., 2009; Subramani lavanya *et al.,* 2011).There are a large number of pesticides currently used in agriculture belonging to a wide variety of chemical classes. Pesticide pollution in water affects the fish and other aquatic organisms, relatively sensitive to changes in their surrounding environment (Ayas et al., 2007). Histopathological analysis appears to be a very sensitive parameter and is crucial in determining cellular damage that may occur in target organs (Altinok *et al*., 2007).

The aim of the present study was to evaluate the histopathological effects of novaluron in gill and liver of *Labeo rohita*. Novaluron is an insecticide for control of listed foliar insect pests on agriculture crops. The primary mode of action is by disrupting cuticle formation and deposition occurring when insects change from one developmental stage to another resulting in death at molting (Nagaraju *et al*., 2011).

MATERIALS AND METHODS

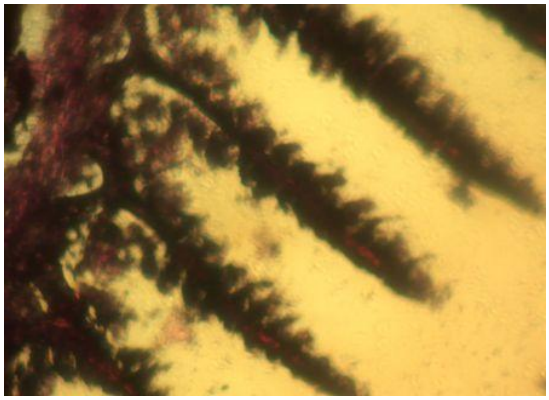

Fig.1.Control gill showing primary gill lamellae and Secondary gill lamellae,100x

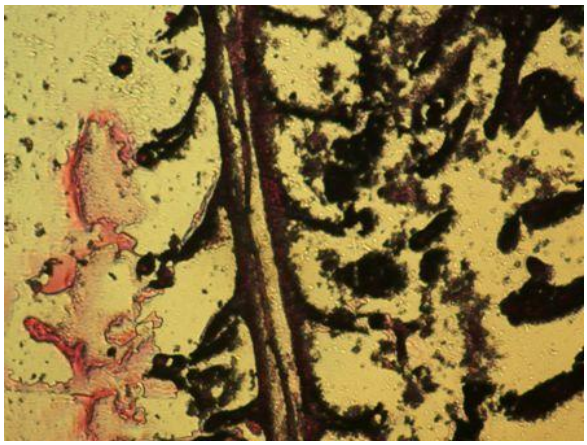

Fig.2. Exposed gill showing Bulging of secondary gill lamellae, epilethial lifting and hyperplasia,100x

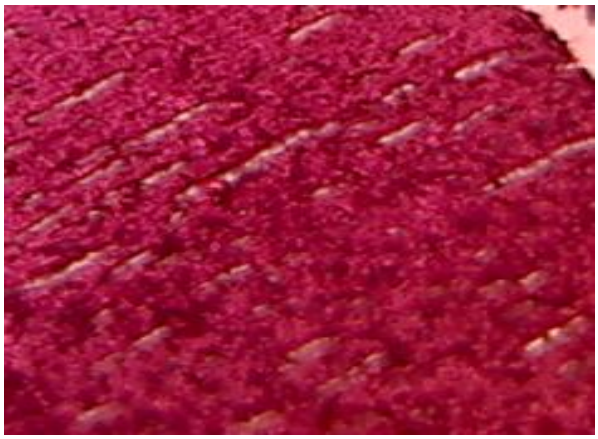

Fig.3.Control liver showing hepatocytes and epithelial cell,100x

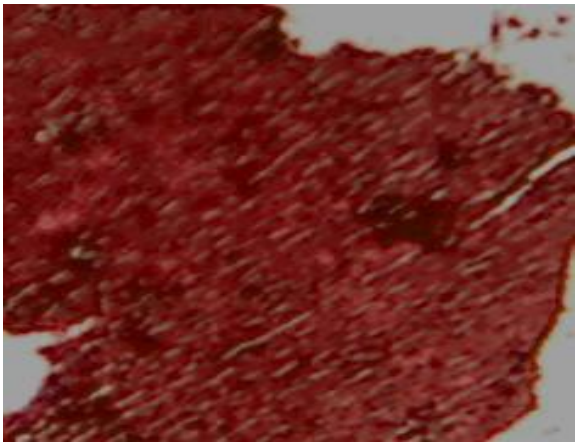

Fig.4.Exposed liver showing pyknotic nuclei,vaculation of Cytoplasm,100x

reshwater fish *Labeo rohita* size 4±6 cm and 8±9 g weight were procured from local fish farm located in Buddham of Guntur, acclimatized to laboratory conditions for 15 days. The containers of the test media are of 15 liter capacity, where in each test five containers were used and each container consisted of 10 fish. The mortality rate was taken into consideration and while taking the data, dead fish was removed immediately. Pilot experiments were conducted to choose the mortality range between 10% and 90%.Basing on the pilot experiments, the experiments were conducted to determine the toxicity in different concentrations 0-16mg/L for 96 h with novaluron, in semi- static system.

The data of each concentration was pooled up to calculate the LC_{50} values. The un-weighted regression method of Probit analysis was used to calculate the LC_{50} values (Probit, 1977,).According to (APHA, 2005) the sample water is clear, colorless and odorless. The 96h LC_{50} value of novaluron to fish was found to be 10mg/L. Fish was exposed for 15 days to sublethal concentrations ($1/10^{th}$ of LC_{50}; i.e. 0.10mg/L) of Novaluron. At the end of the exposed period, fish were randomly selected for histopathological examination.

Tissues like gill and liver were isolated from control and experimental fish. Physiological saline solution (0.85% NaCl) was used to rinse and clean the tissues. They were fixed in aqueous Bouin's solution for 48 hours, processed through graded series of alcohols, cleared in xylene and embedded in paraffin wax. Gills alone were processed by double embedding technique. Sections were cut at 6µ thickness, stained with Ehrlich hematoxylin Eosin (dissolved in 70% in alcohol) (Humason, 1979) and were mounted in *Canada balsam*. The slides were viewed through microscope and photographs were taken with Canon camera.

RESULTS AND DISCUSSION

All the observed changes in the present investigation indicate the irreparable damage to vital organs of the fish exposed to sublethal concentrations of Novalron making it less fit for better survival. Histopathological changes in the gills and liver of fishes due to pesticide chemicals and other environmental contaminants have been reported earlier (Ladipo *et al*., 2011).Histological analysis is sensitive and effective tool to determine cellular change that may occur in vital organs, liver, gills and kidney are suitable organs for histological examination (Capkin,2006).

In the present study the observed changes are excessive mucus secretion, epithelial lifting, aneurysm and lamellar fusion. (Fig.2), no changes were observed in the gills and liver of control fish (Fig.1&3). Toxic effects of glyphosate herbicide on Tilapia was investigated by (Ayoola, 2008), filament cell proliferation, lamellar fusion, lamellar cell hyperplasia and epithelial lifting were observed. The major effects observed on the gills were Oedema, epithelial lifting, and thickening of the primary lamellar epithelium and fusion of secondary lamellae.

Liver exhibits severe necrosis and oedemas were observed in novaluron exposed fish (Fig.4). None of these lesions in gills and liver of control fish. In this present investigation gills were found to be the most seriously affected organ compared to liver, because of the direct contact with the toxicant. Also the liver is an important organ which breaks down chemicals and as a result, liver cells are often among those that are damaged by pesticides etc, showed the fibrosis, large necrosis area, leukocyte infiltration, and the absence of melanomacrophages in the liver (Miranda *et al*., 2008).

Teleosts have five pairs of gill arches. The primary gill filamentous in each arch form two rows and joined at the base by a gill septum. The primary gill lamella (PGL) is flat leaf like structures, with a central rod like supporting axis (CA) with a row of secondary gill lamella (SGL) on each side of it. They are situated laterally on either side of interbrachial septum. The secondary gill lamella is also known as respiratory lamella (Tilak *et al*., 2005). The surface is covered with simple squamous epithelial cells separated by mucous cells erythrocytes and is highly vascularised. Blood vessels can been seen extended into each secondary gill lamella. The blood cell has a single nucleus, which is flattened in appearance (Kiernan, 1990).

The surface of liver is covered with serous membrane and some connective tissue extends inwards into parenchyma. It is composed of parenchymal cells, hepatic cells and lattice fibers, which support the former. Hepatic cells are roundish polygonal, containing clear spherical nucleus. They are located among sinusoids forming cord like structures known as Hepatic cell cords. In fish these structures are generally obscure. Bile canaliculus is centrally located in each cord, fairly large quantities of lipid and Glycogen granules are also observed in the cytoplasm of fish hepatic cells (Kiernan, 1990).

Hepatic cells have many vital functions other than the secretion of bile. They play an important role in protein, lipid and carbohydrate metabolism. They serve as storage sites for some nutrients. Detoxication is another important function. Novaluron has induced discrete pathological changes in the liver tissues. These changes include degenerations of cytoplasm in hepatocytes, atrophy, and necrosis.

Inbamani and Sreenivasan (1998) reported that the histopathological studies on gill, liver and thyroid after the treatment of *Sarotherodon mossambica* with phosphamidon and phosphamidon in thiourea medium revealed that the hypothyroidism has decreased the tolerance of the fish to the pesticide phosphamidon. The effects of Phosphamidon on the gills of *S. mossambica* were observed marked degeneration of cells in the gills, the primary lamella have become thicker, the secondary lamellae were not identical in all regions because of erosion due to pesticides.

According to previous studies,Vardhani and Gouri (2002) the histopathological changes in liver and kidney of *Labeo rohita* intramuscularly injected with B.S.A. pathogeny was evident by the destruction of hepatic cords and tubular cells of the kidney in experimental animals. Tilak *et al*., (2005) observed that chloropyriphos caused marked pathological changes in the gills of exposed fish *Labeo rohita*.

In the present investigation freshwater fish *Labeo rohita* exposed to sublethal concentrations of novaluron caused marked pathological changes of the vital organs like gill and liver of exposed fish. They include atropy, vascular degeneration, cloudy swelling, bulging in the tips of primary gill lamellae, club shaped secondary gill lamellae and severe necrotic changes in the epithelial tissues of secondary gill lamellae. Novaluron induced discrete pathological changes in the liver tissues. These changes include degeneration of cytoplasm in hepatocytes, atropy, necrosis, formation of vacuoles, rupture in blood vessels and disappearance of hepatocytic wall and disposition of hepatic cords.

All the observed changes in the present investigation indicate that the pesticide Novaluron is causing irreparable damage to the fishes. While pesticide is desirable for pest control, the unprecedented usage of these compounds causes untold damage to the non-target organisms. The indiscriminate use of these pesticides cause biochemical, histopathological and histochemical changes may alter the normal living conditions of the fish and thereby bring down the health and production.

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