INDOXACARB INDUCED HISTOPATHOLOGICAL CHANGES IN GILL OF FRESHWATER BIVALVE, *PARREYSIA CYLINDRICA*

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ABSTRACT

Freshwater bivalves were exposed to acute and chronic dose of indoxacarb so as to study the histopathological effects of pesticide on gills in freshwater bivalve, *Parreysia cylindrica*. Severe damages were found in gills of pesticide exposed bivalves. The gills were severely damaged showing swollen tips of filaments, damage of chitinous rods, air spaces, vacuolization and detached gill filaments in the pesticide exposed bivalves. In the bivalves exposed pesticides, the normal structure of the hepatic follicles was lost, connective tissue between the follicles was affected, and the hepatic secretary cells and the lumen were also affected. Intensity of damage in the tissues of pesticide exposed bivalves was found to be increased with increase in exposure time.

INTRODUCTION

Pollution stress due to pesticides in aquatic animals has attracted attention of scientists all over the world. The increasing use of pesticides to increase the agricultural production to match the explosive population growth rate is a global phenomenon (Srivastava et.al, 1977). Pesticides damage the functioning of aquatic organisms even at low concentration. These sub lethal concentrations of pesticides influence the biochemical, physiological, behavioral functioning and life cycle of aquatic organisms. Small change in environment due to pollution may have ecological importance, may be in lowering the fitness of organism (Sprague, 1971). Respiratory organ is gill in aquatic animals which always came in contact with the surrounding water. If water is polluted with pollutant like pesticides that polluted water affect the function of gills. These pollutants affect the structure of the respiratory organs.

Incorporation of toxic compounds even at very low levels in lower organisms and in the vital tissues of fishes, birds and humans have been recorded to cause serious morphological alterations (Chakrabarthy and Konar, 1974; Mathur et. al., 1981). Structural and functional changes which occur in the tissues of animals due to different toxicants. In order to understand a pattern of damage caused by pollutant to the tissue, it is essential to study the histological analysis of the tissues. This can be useful for better understanding of the pathological condition and damages of tissues under toxic stress of pesticides. Histopathological study is an extremely useful tool for assessing effects of toxicants at individual level and also useful to find out the exact location of the action of pollutants in the various organs and systems of animals. The histopathological study is important in identification of specific cell, tissues and organs that have been affected in the form of injury. Histological studies of bivalve exposed to pesticides shown that organs of mollusc are efficient indicator of water quality (Muley and Mane 1988; Thoser et.al., 2001). The gills are vital organs to perform respiration, osmoregulation, acid base balance and nitrogenous waste excretion (Health 1987). The gills are exposed to pollutants in water because of their large surface area and having a key role in the transport of oxygen for the metabolic activities, they offer, a favourable material for studies on effects of pollutants on respiration. Therefore, Swartz (1972) recommended the studies of gill histopathology to understand the biological response of aquatic animals to a variety of aquatic pollutants. Histological studies on gills of the bivalve, *Corbicula striatella* after exposure to pesticides, carbaryl, endosulfan and cypermethrin were made by Jadhav (1993). Waykar and Lomte (1998) studied histological studies of gills of the bivalve, *Parreysia cylindrica* after acute and chronic exposure to pesticides.

Investigation regarding the histopathological impact of pesticides on tissues of freshwater bivalve is scarce therefore, in present work efforts are taken to observe effects of pesticides indoxacarb on histopathological changes in gills of freshwater bivalve, *Parreysia cylindrica*.

MATERIALS AND METHODS

The fresh water bivalve, *Parreysia cylindrica* were collected from the Jamda dam which is nearly 30 Kms away from Chalisgaon, Dist. Jalgaon (M.S). After collection, the bivalves were acclimatized in the laboratory condition at room temperature for 4-6 days. The active acclimatized bivalves of approximately same size were selected for experimentation. Before starting the experiment, these bivalves were divided into five groups such as A, B, C, D and E. 1. 'A' group of bivalves were maintained as control. 2. 'B' group of bivalves were exposed to sub lethal dose (0.3905ppm $LC_{50/2}$ of 96 hrs) to indoxacarb upto 24 hours. 3. 'C' group of bivalves were exposed to sub lethal dose (0.3905ppm $LC_{50/2}$ of 96 hrs) to indoxacarb upto 96 hours. 4. 'D' group of bivalves were exposed to chronic dose (0.07811ppm $LC_{50/10}$ of 96 hrs) to indoxacarb, upto 7 days. 4. 'E' group of bivalves were exposed to chronic dose (0.07811ppm $LC_{50/10}$ of 96 hrs) to indoxacarb, upto 21 days. The control and experimental bivalves of A, B and C groups were dissected after 24 and 96 hours and animals from D and E group of chronic treatment after 7 days and 21 days. Their gills were removed and fixed in Bouin's fluid for 24 hrs, washed and dehydrated in alcohol grades, cleared in toluene and embedded in paraffin wax (58-60^0 C). Prepared blocks of tissues were cut at the thickness of 5µ to 6µ and stained with Mallory's triple stain. Stained sections of gills of bivalves from all groups i.e. control and treated were screened to study the effect of pesticide and the effect is presented through the photomicroplates for comparison.

OBSERVATIONS AND RESULTS

Histopathological disorders were observed in the gill of fresh water bivalve, *Parreysia cylindrica* after exposure to indoxacarb. Many investigators have reported toxicant induced histopathological changes in certain tissues of various animals (Goel and Garg, 1980; Banerjee and Bhattacharya, 1997). In bivalve, the gill is the most important organ for respiration and it is the first organ, which comes into contact with the pollutant first.

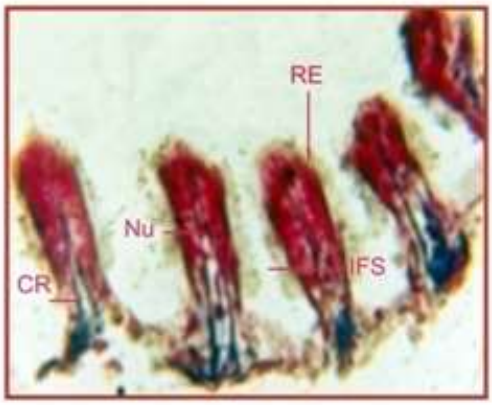

Fig. (a)

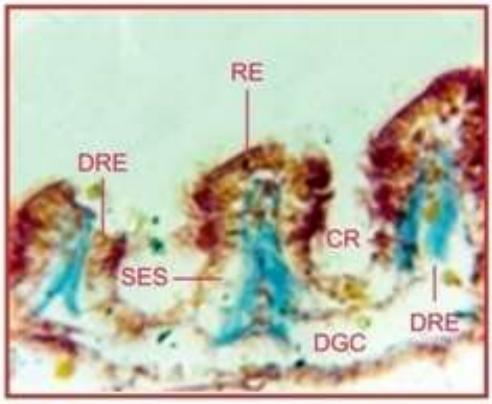

Fig. (b)

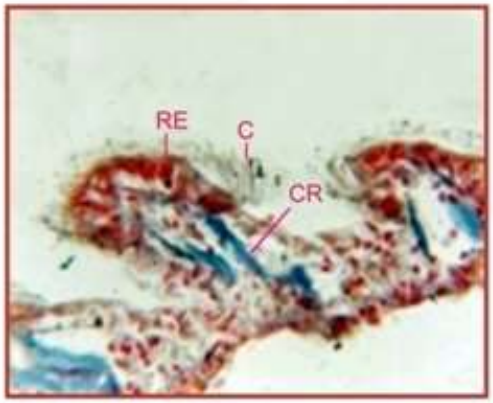

Fig. (c)

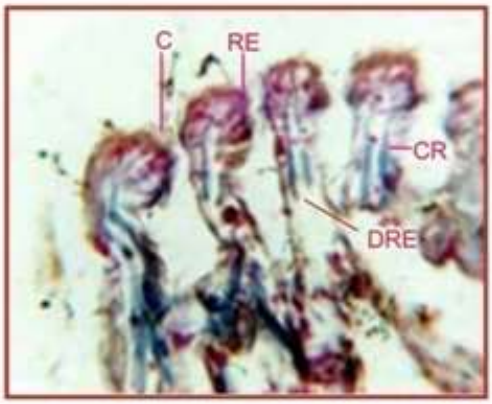

Fig. (d)

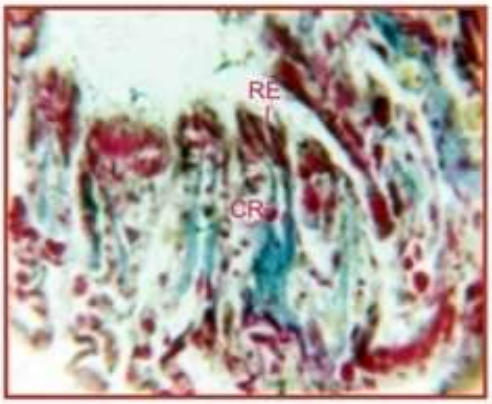

Fig. (e)

Microphotographs of longitudinal section of gill of bivalves, *Parreysia cylindrica* on acute and chronic exposure to indoxacarb (X400). Fig. a- L.S.of gill of control bivalve Fig.b-L.S.of gill from bivalve exposed up to 24 hours. Fig.c - L.S.of gill from bivalve exposed up to 96 hours. Fig.d - L.S.of gill from bivalve exposed up to 7 days. Fig.e - L.S.of gill from bivalve exposed up to 21days. Abbreviation: C - Ciliary border CR - Chitinous rods IFS - Inter filamental space Nu - Nucleus RE - Respiratory epithelium DRE -Degenerating respiratory epithelium DGC -Degenerating gill cell SES - Sub epithelial space.

There is increasing evidence that toxicants have a potential to cause the most harm to organs that contact first (Timbrell; 1991). The reason is that gills are main absorption place for the toxic compounds. Hence, it is more susceptible to damage than any other tissue. Microphotography Fig. a shows normal gill lamellae with stratified epithelial cells, normal water space in between two secondary gill lamellae, normal blood capillary and supporting chitinous rods. Gill histopathology on exposure to various duration to acute and chronic treatment of indoxacarb were presented in Figure b to e. As compared to control gills of bivalve, *Perreysia cylindrica*, after acute exposure (0.3905 PPM) to indoxacarb has induced marked pathological changes in gill structure, characterized by swelling, epithelial necrosis, degeneration and vacuolization in respiratory epithelial cells and connective tissue core. At 24 hours of exposure the bases of secondary gill filaments become broader and sub epithelial spaces were found at respiratory epithelial level. Most degenerative changes were noted in respiratory epithelial cells. Necrosis at ciliary's epithelial cells were noted because of which vacuoles were formed at the tip of secondary gill lamellae. Supporting chitinous rods were damaged (Fig. b). The histopathological changes were gradually increased as exposure period was increased. After 96 hours of exposure, overall elongation of secondary gill filaments was observed. In some places of secondary gill lamellae, lamellar detachment of the epithelial cells along with damaged chitinous rods was observed. Large irregular spaces were found in the connective tissue core. Necrosis and vacuolization was very common in secondary gill lamellae and connective tissue. Progressive damaged chitinous rods were observed with increase in exposure periods, resulting in abnormal shape of gill filaments. In the gill lamellae shrunken blood cells were seen. These cells were scattered in gill lamellae and connective tissue (Fig. c). After chronic exposure of indoxacarb (0.07811 PPM), at 7 days of exposure, elongation of secondary gill filament with bulging and bending at their tips and degenerative changes at distal ends were observed. At connective tissue level most prominent effect observed was degeneration, necrosis and vacuolization. Supporting chitinous rods of gill filaments were damaged at certain places (Fig. d). Elongation of secondary gill filament with bulging and bending tips and

degenerative changes at distal ends were observed after 21 days of exposure. At the proximal end atrophy, clubbing and fusion of neighboring secondary gill filaments and at the distal end necrotic changes were observed. Severe gill lesions such as necrosis, vacuolization and dissolution of respiratory epithelial cells of secondary gill lamellae were also observed. Supporting chitinous rods were damaged. Detached secondary gill lamellae were seen. Overall disintegration of gill lamellae was seen (Fig. e).

Mattiessen and Brafield (1973) reported necrosis of gill epithelium, vacuolization and sloughing of epithelial cells with changes in their cytoplasm in gills due to zinc toxicity of sickle backs, *Gastersteus faculeatus*. Jadhav (1993) reported hyperplasia, degeneration of respiratory epithelial cells, and fusion of secondary gill lamellae, vacuolization in respiratory epithelium and connective tissue core and damage of chitinous rods after exposure to pesticides of gill lamellae of bivalve, *Corbicula striatela*. Reduction in the size of primary and secondary gill lamellae and necrosis of tissue, degeneration of secondary gill lamellae with bulging tips also found. Hughes and Morgan, (1973) reported thickening of gill epithelium is also one of the earlier morphological changes that follow exposure to pollutant. Epithelial thickening has been attributed to cell swelling and usually followed by fusion of adjacent lamellae. Kshemkalyani et. al., (1990) observed the swelling of secondary gill lamellae at the tips, fusion of adjacent secondary gill lamellae, haecytic congestion, necrosis and hyperplasia cell in *Lepidocephalus guntae* after pesticide exposure. In indoxacarb exposed bivalve, epithelial necrosis, degeneration and vacuolization in respiratory epithelial cells and connective tissue core, elongation of secondary gill lamellae with bulging and bending at their tips and degenerative changes in the respiratory epithelial cells were observed. Damage of skeletal rod were noted after acute and chronic exposure to indoxacarb (Fig. b to e). Waykar and Lomte (1998) reported, swelling, degeneration, necrosis, vacuolization in respiratory epithelium and elongation of secondary gill filaments with bulging and bending at tips, clubbing and fusion of neighboring secondary gill filaments, damaged chitinous rods in bivalve after exposure to carbaryl. These histopathological alterations reduce the respiratory area there by reducing the respiratory and osmoregulatory potential.

It also indicates a reduce in energy metabolism due to degeneration of respiratory epithelium and the damage of the gill tissue may finally result in tissue hypoxia. These pathological changes in respiratory gill might have resulted in shift from aerobic to anaerobic pathway in tissue under pollutants stress. Damage to the epithelium during any sort of pollutant poisoning may affect gas exchange, extra renal excretion or ion exchange. Osmotic and ionic imbalance is ruled out as a main cause of the death (Hughes and Morgan, 1973). Mallat (1985) in an exhaustive review of toxicant induced alterations in the gills showed that such changes tend to be largely non-specific, and seem to reflect physiological adaptation to stress. Epithelial hyperplasia resulting in lamellar fusion and clubbing of secondary gill lamellae, could be seen as defensive response (Smart, 1976), against prolonged exposure to contaminant. Epithelial lifting and lamellar fusion were also suggested as defensive measure by decreasing the vulnerable surface area of the gills, to maintain its osmoregulatory function while maintaining a progressive loss of its basic functions (Abel, 1976). However such reaction that helps slow down toxicant uptake could result in dysfunctional gills, and eventually asphyxiate the bivalve. It is important to mention here that the morphometric study showed that the surface area of gill was particularly decreased with increasing exposure period. The observed fusion of adjacent lamellae and epithelial cells would affect oxygen uptake by decreasing surface area. From present study it is clear that the pesticide intoxication must have severely changed the membrane permeability of the gill epithelia resulting in to inflow of water and caused swelling of the epithelial cells. In the present investigation disappearance of supporting skeletal rods at certain places is observed. Similar results were reported in mollusk by (Waykar and Lomte 2002; Waykar, 2007) after exposure to pesticide and suggested that the sever pesticide toxicity caused disintegration of the structure of supporting rod of the gill as a whole, resulting into the abnormal shape of gill filaments. From the results obtained in the present study it is clear that, acute and chronic treatment of pesticide indoxacarb badly affected the normal structure of gill and increases period of exposure of pesticide caused an increase in structural damage and developed different pathological symptoms in the bivalve *Parreysia cylindrica*.

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