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AG Patil
Shri V. S. Naik Arts, Commerce and Science College, Raver, Jalgaon, Maharashtra, India

Corresponding Author:
AG Patil
Shri V. S. Naik Arts, Commerce and Science College, Raver, Jalgaon, MS, India

Thiamethoxam induced histopathological alterations in hepatopancreas of freshwater bivalve *Parreysia cylindrica*

AG Patil

Abstract
Freshwater bivalves, *Parreysia cylindrica* were exposed to acute and chronic dose of thiamethoxam so as to study the histopathological effects of pesticide on hepatopancreas. The histopathological studies have shown that the thiamethoxam has affected the hepatopancreas when compared with their respective control. Severe damages were found in hepatopancreas of pesticide exposed bivalves. Intensity of damage in the tissues of pesticide exposed bivalves was found to be increased with increase in exposure period.

Keywords: Bivalves, *Parreysia cylindrica,* thiamethoxam, hepatopancreas

Introduction
Pollution stress due to pesticides in aquatic Animals has attracted attention of scientists all over the world. The increasing use of pesticides in order to enhance the agricultural productivity to match the explosive population growth rate is a global phenomenon (Srivastava *et al.*, 1977) [19]. Pesticides impair the functioning of organisms in aquatic environment even at low level. These sub lethal concentrations of pesticides affect the physiological, biochemical, behavioral functioning and even life cycle of organisms. Small change in environment by pollution may have ecological importance, may be in lowering the fitness of organism in its environment and not merely within the range of adaptation of organism (Sprague, 1971) [18].
For the better survival of the life of animals required all the systems work coordinately. Digestive systems play most important role in the life of animal because efficiency of the animals depends on it. For proper working of digestive system, alimentary canal and digestive gland should be healthy. Most of the pollutant enters in digestive system through the water or food material and affecting the alimentary canal and digestive glands. Due to unhealthy digestive glands food digestion does not occur properly ultimately affecting the health of animal.
Haepatopancreas is most important digestive gland of mollusc, where detoxification also takes place. So toxic material first circulated by animal through the haepatopancreas. If small amount of toxicant enters in the body that can be detoxicated but if more toxic substance enters in the body of animals then it affects the structure and function of the digestive gland. As energy produced by digestive gland cells is used for detoxification hence sufficient energy is not available to gland cells to secrete the proper enzymes and hence affects the digestion. Pesticide alters the structure and physiology of the digestive gland due to stress. Disorder in the digestive gland affects all metabolic activities. Structural and functional changes which occur in the different tissues of animals due to different toxicants vary from tissue to tissue. To understand a pattern of damage caused by particular toxicant to the tissue, it is essential to have an insight into the histological analysis of the tissues. This can be useful for better understanding of the pathological condition, abnormalities and damages of tissues under toxic stress of pesticides. Thus histopathology is a very useful tool for assessing effects of toxicants at individual level.
Histopathological study is also useful to find out the exact location of the action of toxicant in the various organs and systems of animals. Intake of the pollutants affects the mollusk behavior, physiology, metabolism and cellular structure. Acute and chronic treatment of pesticides causes histopathological changes in digestive gland of mollusc (Jonnalagadda and Rao, 1996; Thoser *et al*., 2001) [11, 23].

Histopathological changes are generally confined to important organs which directly involved in their metabolism and detoxification (Rashatwar and Ilyas, 1994) [16]. Hepatopancreas is the main site of action in bivalves, degradation and detoxification of pesticide takes place in hepatopancreas, hence hepatopancreas were chosen as target organ.

Histopathological changes in the fish hepatopancreas due to industrial pollutants and chlorinated hydrocarbons was studied by Dubale and Shah (1979) [9]. Amminikutty and Rege (1977) [1] observed the effects on the liver of widow tetra *Gymnocorymbus ternetzi* exposed to acute and chronic treatment of pesticides, thiodan and agallol. Annes (1978) [2] studied hepatic pathology in a freshwater teleost *Channa punctatus* exposed to sublethal and chronic treatment of three organophosphorous insecticides. Usheva *et al.*, (2006) [24] studied histopathology of the digestive gland of bivalve mollusc, *Crenomytilus grayanus*.

Informations regarding the histopathological impact of pesticides on tissues of freshwater bivalve are scarce therefore, in present work efforts are taken to observe effects of pesticides thiamethoxam on histopathological alterations in hepatopancreas 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 (14.2114ppm $LC_{50/2}$ of 96 hrs) to thiamethoxam upto 24 hours. 3. 'C' group of bivalves were exposed to sub lethal dose (14.2114ppm $LC_{50/2}$ of 96 hrs) to thiamethoxam upto 96 hours. 4. 'D' group of bivalves were exposed to chronic dose (2.8422ppm $LC_{50/10}$ of 96 hrs) to thiamethoxam, upto 7 days. 4. 'E' group of bivalves were exposed to chronic dose (2.8422ppm $LC_{50/10}$ of 96 hrs) to thiamethoxam, 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 digestive glands 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 digestive glands 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.

Results and Discussion

Histopathological disorders were observed in the hepatopancreas of fresh water bivalve, *Parreysia cylindrica* after exposure to thiamethoxam. Many investigators have reported toxicant induced histopathological changes in different tissues of various animals (Goel and Garg, 1980; Banerjee and Bhattacharya, 1997) [10, 4].

Hepatopancreas is also known as the digestive gland it is composed of tubules of different shapes and sizes. Microphotography (Fig. a) shows the tubules are surrounded by the intertubular connective tissue with some muscles, collagen fibers and amoebocytes. The epithelium of the tubule is placed on a thin basement membrane. The epithelial cells have basally situated nuclei. Tubular epithelium comprises of the digestive and the secretary cells. The digestive cells are highly vacuolated and elongated, roughly cylindrical with the spherical nucleus at the base. Granular material may be present in the vacuolar cytoplasm. The more or less pyramidal cells are known as the calcium cum secretary cells, normally appear triangular with varying heights. Their cytoplasm is more or less homogenous, having a conspicuous nucleus, which is much bigger than that of digestive cells. The selective calcium staining shows that the brown pellets which are so big, most of the cells containing the nucleus pushed aside to the basal part of the cells. These calcium spherules occupy a basal position in the cells. Presences of such calciferous cells have also been reported in the hepatopancreas tubules of viviparous by Thile (1953) [22].

Hepatopancreas histopathology on exposure to various duration to acute and chronic treatment of thiamethoxam were presented in Figure b to e. As compared to control animal's hepatopancreas of bivalve, *Parreysia cylindrica* after acute exposure to thiamethoxam (14.2114PPM), marked histopathological changes were induced. This exhibited an initial reaction of epithelial hyperplasia, together with necrotic changes in basement membrane and intertubular connective tissue. In present study it was also observed the epithelial necrosis, rupture of epithelial layer, hypertrophy and sloughing of the epithelium were noted at 24 hours exposure (Fig. b). The severity of damage of hepatopancreas progressed with longer exposure. After 96 hours of exposure to thiamethoxam tubular hyperplasia is seen which were transformed into globular or balloon like structure resulting into rupture and discharge of secretary products from secretary cells and flattening of the epithelial cells along with displacement of nuclei and widening of tubule lumen. The nuclei become enlarged than the normal ones. Necrosis of the tubules and basement membrane at some places were observed (Fig. c). The result of the microscopy shows that epithelial tissue is probably a primary target of the pesticide intoxication. Drastic pathological changes in the structure of hepatopancreas of bivalve, *Parreysia cylindrica* were observed, after chronic exposure to [2.8422PPM] along with effect of acute exposure epithelial hyperplasia was noted because of which tubular lumen was reduced. At certain places basement membrane was interrupted. Thickening of tubules was evident. Some epithelial cells burst out due to hyperplasia. At certain places epithelium showed dissolution at 7 days of exposure (Fig. d). After 21 days of treatment, the effect is more pronounced. In the tubule, the epithelial cells were seen separated from the basement membrane. The cellular identity of cells was found to belost. Calciferous deposition is found to have increased in the cells. The inter tubular mass was loosely arranged (Fig. e).

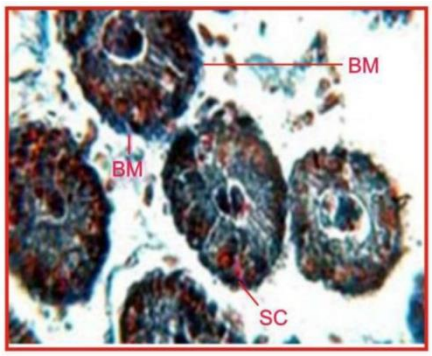

Fig. (a)

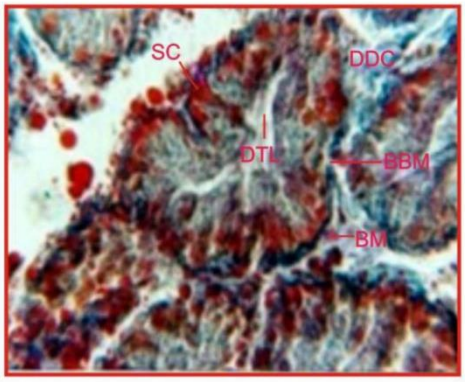

Fig. (b)

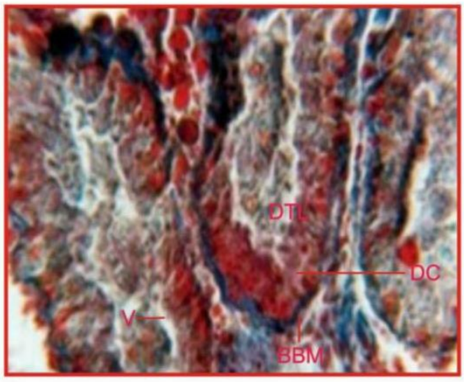

Fig. (c)

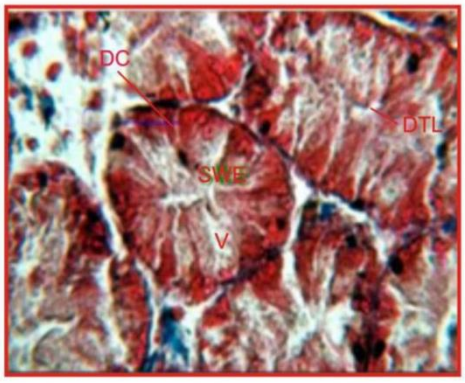

Fig. (d)

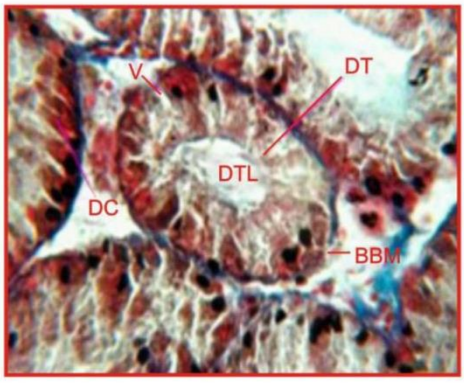

Fig. (e)

Microphotographs of longitudinal section of hepatopancreas of bivalves, *Parreysia cylindrica* on acute and chronic exposure to thiamethoxam (X400).

Fig.a- L.S.of hepatopancreas of control bivalve **Fig. b -** L.S.of hepatopancreas from bivalve exposed up to 24 hours. **Fig.c-**L.S.of hepatopancreas from bivalve exposed up to 96 hours **Fig. d-** L. S. of hepatopancreas from bivalve exposed up to 7 days. **Fig.e-**L.S.of hepatopancreas from bivalve exposed up to 21days

Abbreviation: SC - Calcium secretory cell **BM -**Basement membrane **DT -** Digestive tube **DC -** Digestive cell **DCT -** Degenerative connective tissue **BBM -** Broken basement membrane **DEC -** Degenerating epithelial cell **DCC -** Degenerating connective cell **DTL -**Digestive gland lumen **SWE -**Swollen epithelial cell **N -** Nucleus **V** – Vacuole

The severity of the pathological changes depends on many factors such as type of pesticides, concentration of dose, time of exposure, type of animal species and on the rate of metabolism in the target tissues. Histopathological changes in the hepatopancreas of *P. hydroaromous* in response to cythion as reduction in the height of tubular epithelium, enlargement of lumen, vacuolization and atrophy was observed by Victor *et al.*, (1990) [26].

Many investigators have reported toxicant induced histopathological abnormalities and degenerative changes invarious tissues of various animals (Goel and Garg, 1980; Banerjee and Bhattacharya, 1997) [4]. Victor *et al.*, (1990) [26] concluded that, the histopathological changes were due to inability of animal to digest and store food properly and hence lack of nutrients resulted in the atrophy of hepatopancreas. Amminikutty and Rege (1977) [1] reported disintegration or partial damage of exocrine and endocrine portions of hepatopancreas of teleost treated with a variety of pesticides.

Many workers (Clements *et al.*, 1980; Axiak *et al.*, 1988; Bright and Ellis, 1989; Mariaomez *et al.*, 1990; Srivastawa and Maurya 1991; Vincente *et al.*, 1998; Pillai and Menon, 1998) [8, 3, 6, 13, 17, 25, 20] studied the effect of different toxicants on hepatopancreas in various aquatic animals. Muley (1990) [14] found severe damages in the gonads and hepatopancreas of fresh water mussel, *Indonaia caeruleus* exposed to fluoride.

In the present study it is also observed that the severity of hepatopancreas damage was increased with longer exposure period to thiamethoxam. Suresh (2001) [21] reported disorganized condition of hepatopancreas in *U. annulipes* in response to cadmium and mercury. Similar histopathological alterations in hepatopancreas were reported by number of workers in mollusk after pesticides treatment (Mane *et al*, 1979; Muley and Mane, 1990; Jonnalagadda and Rao, 1996; Thoser *et al.*, 2001; Waykar, 2007) [12, 15, 11, 23, 27].

Birgul *et al.*, (2004) [5] observed amoebocytes infiltration, dilatation in haemolymphatic spaces between the tubules, degeneration of cells, abnormal lumen, necrosis of cells and atrophy in the connective tissue of digestive glands of snail, *Planorbarius corneus* exposed to endosulfan. Cengiz *et al.*, (2005) [7] also observed accumulation of amoebocytes in the haemolymphatic spaces between the tubules of the digestive glands, exudation in the lumen of tubules, expansion of haemolymphatic spaces between the tubules and increase of vacuolization and necrotic changes in digestive glands of freshwater snail, *Galba truncatula* exposed to thiodan.

From the results obtained in the present study it is clear that, acute and chronic treatment of pesticide thiamethoxam badly affected the normal structure of hepatopancreas and increases period of exposure of pesticide caused an increase in structural damage of hepatopancreas in the bivalve, *Parreysia cylindrica*.

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