Histopathological Studies on the Gill and Liver Tissue of Etroplus maculatus Exposed to Fluben Diamide

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Abstract

Pesticides have been one of the most effective weapons discovered by man to protect agricultural products from the attack of pests. As the results of the survey conducted among the farmers and fishermen of the lower Kuttanad area, Fluben Diamide is found to be a widely used pesticide in the paddy fields of Kuttanad, ‘the rice bowl’ of Kerala. Etroplus maculatus is said to be an important freshwater teleost food fish inhabiting the intricate canals and polders of Kuttanad. So, in the present study, an attempt has been made to estimate the lethal toxicity of the pesticide Fluben Diamide on the juveniles of fish Etroplus maculatus, and to analyse the sublethal effect of the pesticide on their Gill and Liver tissue. The histological changes observed in all the tissues of E. maculatus indicate that sub lethal concentrations caused moderate to severe alteration in gill and liver which are important organs performing vital function like detoxification, respiration etc. The application factor derived from the laboratory studies can be directly applied to the field situations, as the fishes are the true inhabitants of Kuttanad.

Keywords: Fluben Diamide, hyperplasia, vacuolization, hypertrophy, necrosis

I. INTRODUCTION

Kuttanadu is a region in the Alapuzha, Pathanamthitta and Kottayam Districts in the State of Kerala, India, with the lowest altitude in India, and one of the few places in the world where farming is carried out below sea level. Kuttanadu is known as the ‘rice bowl’ of Kerala as it is rich in paddy cultivation. A variety of fertilizers, herbicides and pesticides are used by farmers for better yield and ultimately all these chemicals are directly discharged into the water bodies in and around the paddy fields. The fishes and other non target aquatic organisms are continuously exposed to these unhealthy or toxic substances dissolved in the medium where they are inhabiting throughout their life in water.

Histopathology deals with the study of pathological changes induced in the microscopical structure of the body tissue. In the present study an attempt has been made to estimate the lethal toxicity of Fluben Diamide on the juveniles of Etroplus maculatus, with a view to understand the extent of tolerance of these fishes to this pesticide The fishes were then exposed to sublethal concentrations of this pesticide for a period of 30 days and the histopathological studies were conducted on physiologically important tissues like gill and liver during the interval of 7th, 14th, 21st and 28th days. The results were compared with that of fishes under controlled conditions.

Histopathological studies have been widely used as biomarkers in the evaluation of the fish exposed to the pesticides, both in the laboratory as well as in the field (Pragna H Parikh et al.,2010; Wester and Canton,1991; Hinton et al.,1993; Schwaiger et al.,1997). One of the great advantages of using histopathological biomarkers in environmental monitoring is that this category of biomarkers allow examining specific target organs like gills and liver, that are responsible for vital functions, such as respiration, excretion, and accumulation and biotransformation of xenobiotics in the fish (Gernhofer et al., 2001); and serve as warning signs of damage to animal health (Hinton and Lauren,1993). Accumulation of pesticides in tissues produces many physiological and biochemical changes in the fishes and freshwater fauna by influencing the activities of several enzymes and metabolites (Nagarathnamma & Ramamurthi, 1982) . Pesticides are also well known for causing more toxic effects in teleost fishes (Muthukumaravel et al., 2013) . Histopathological alterations could be used as indicators for the effects of various anthropogenic pollutants on organisms and are a reflection of the overall health of the entire population in the ecosystem (Mohamad, 2009). Hence the present study is aimed to look into the histoarchitectural alterations in Fluben diamide induced toxicity in some of the vital organs like gill and liver of the teleost fish, Etroplus maculatus, so as to assess the damage and get an insight into their functional consequences.

II. MATERIALS AND METHODS

Juveniles of Etroplus maculatus (3.5 - 4.5 cm in total length and 2.5 - 4gm in weight) were collected from the Kuttanad area with the help of big nylon net and hand net. They were brought to the laboratory and acclimatized to the laboratory conditions for a
period of 10 days prior to the experiment. During this period, they were fed, once a day, on pelleted fish feed and were kept aerated (Prashanth, 2011). Each trough contained 15 litres of water with uniform number of fishes. After 10 days, fishes with normal behavioural activity and good health conditions were selected for further experiment purpose. After exploratory tests, five concentrations of Fluben diamide from 450 ppm (no mortality) to 650 ppm (100% mortality) were chosen for final 96 hour test to determine the 50% lethal concentration (LC50). Static bioassay with toxicant replenishment every twenty four hours interval, was carried out in triplicate in containers with five litres of water and six fishes each. A control without any contamination of pesticide was also kept. The physicochemical parameters were also measured (oxygen 6.5 - 7.5 mg/litre; temperature 27 ± 1°C; pH 6.5- 7). Mortality during the 96 hour exposure period was recorded for every 12 hr interval. The LC50 value for 96 h was determined by probit analysis method (Finney 1971).

The fishes were divided into two groups. Group I fishes were not exposed to pesticide and served as control. Whereas, group II fishes were exposed to sub lethal concentrations of Fluben diamide for 30 days. And fishes were chosen on 1st, 7th, 14th, 21st and 28th day to observe the short-term and long term effects respectively. Water was renewed after every 24 h to maintain the pesticide concentration. At the end of experiment periods, viz; on 7th, 14th, 21st and 28th days of exposure to sublethal concentrations(less than 1/4th of the LC50 value,ie,125ppm) of Fluben diamide, fishes were captured and sacrificed. Then, they were dissected and tissues like gill and liver were collected and washed by buffered normal saline. Tissues were fixed in Bouin’s solution (prepared with saturated picric acid, formaldehyde and acetic acid), for 48 h and then dehydrated through graded alcohol series (70 to 100%), cleared in xylene and embedded in paraffin. 5 μm thick paraffin sections were cut, ribbon of sections were taken on slides and excessive wax was removed by heating slide on lamp. The sections were stained with haematoxylin-eosin, investigated and analyzed under a light microscope. Histological alterations induced in the tissues by treatment were analyzed and photographed by light microscope at 10X eye piece magnification and 40X objective magnification using Labomed (Model: Vision-2000) with camera attachment.

III. RESULTS

The study revealed the 96h LC50 value as 525 ppm with confidence limits of 494.236ppm and 555.764ppm. The treated group showed an insignificant reduction in body weight. Abnormal behaviour such as restlessness, sudden quick and jerky movements, were observed in the fishes exposed to shorter duration , whereas, increased opercular movements accompanied with surface to bottom movements and loss of equilibrium were observed in the fishes exposed to longer duration. Similar observations have been reported by Hinton et al.,(1993) and Raheman et al.,(2002) with various organophosphate insecticides.

Effect of Fluben diamide on Gill:

In teleost fishes, gills are critical organs which perform respiratory, osmoregulatory and excretory functions and the first target of waterborne pollutants due to the constant contact with the external environment (Camargo and Martinez, 2007).The histology of gill in E. maculatus under controlled conditions is shown in Plate 1 Fig A. Each gill is made up of two rows of gill filaments and is attached to the opposite sides of the inter branchial septum. Each gill filament is supported by a skeletal axis, consisting of epithelial cells, secondary lamellae and pillar cells. Filaments have a central cartilaginous support, afferent and efferent arterioles and a thin epithelial covering. The primary lamella is a fold of epithelium and consists of a central vascular core. Secondary lamellae originate on both the surfaces of primary lamellae and are oriented perpendicular to the filaments. The secondary lamellae possess numerous channels of blood capillaries. The thin epithelial covering of the secondary lamellae lies on a basement membrane supported by pillar cells.Different mucous cells are scattered throughout the normal gill epithelium of fresh-water fishes, mainly towards the tip and base of the filaments. The secondary lamellae of the gill filaments are the sites of gas exchange in fish gills. They are mainly composed of two epithelial sheets joined together by pillar cells. These cells are characterized by collagen columns contained in infoldings of the cell membrane and oriented perpendicular to the epithelial sheets. The gill is the first organ to which the blood flows from the heart and within the secondary lamellae it flows through channels between the pillar cells. In addition to these cells, there are other cells with the nucleus stained red, and the cytoplasm blue and granulated in appearance. Some of these cells are oval and some are round or irregular in shape. The nucleus of the cell is situated towards the centre of the cell. The histopathological studies indicated that the gill of E.maculatus were affected by sub lethal concentrations of Fluben diamide (Takumin).

After 7 days (Plate 1fig B), minimum changes were observed, including the thickening of the gill filaments and the epithelial hyperplasia. After 14 days, lifting of epithelial cells were observed in the primary lamellae but the secondary lamellae were fused together to most of their length with the terminal 1/3 portion free (Plate 1fig C). The infused secondary lamellae were thinner compared to their controls. The epithelial cells were seen in between the fused secondary lamellae. In some regions, secondary lamellae appeared to be curved. In 21 days treated fish, pronounced changes like distortion of secondary lamellae, hypertrophy of gill filament and hyperplasia of epithelial surface, detachment of epithelium from underlying pillar system and the fusion of secondary lamellae resulting in a reduction in the surface area of the epithelium were noticed (Plate 1fig D). In fish treated upto 28 days, the changes observed in the gill of E.maculatus were severe erosion and degeneration of gill epithelium and aggregation of blood corpuscles, hypertrophy, hyperplasia, necrosis of the epithelial cells, epithelial lifting, degeneration and necrosis of epithelial cells, disruption of epithelial cells from pillar cells, complete destruction of lamellae, increased vacuolization and irregular appearance of gill lamellae(Plate 1fig E, F,G & H).Such degenerative changes in gill, such as intraepithelial edema in the secondary lamellae, erosion of secondary lamellae, thickening of lamellae, inflammation of epithelial cells, degeneration of secondary lamellae, necrosis, rupture of epithelium were noticed during exposure to sublethal
concentrations of monocrotophos by Rao et al. (2005). The gills of fish exposed to 14 days of exposure showed curling and clubbing of secondary lamellae. Enlargement of primary lamellae and loss of secondary lamellae were seen at later days of exposure. (Plate 1Fig. D & E). These pathological changes may be a reaction to toxicant intake or an adaptive response to prevent the entry of the pollutants through the gill surface and probably due to increased capillary permeability. Gill is one of the most important organs directly in contact with pollutants and any kind of damage to the gill tissue of fish leads to disorder in the gas exchange process and also the decrease of ion regulation efficiency via this organ (Fernandes, M. N. and A. F. Mazon, 2003). Histopathology of gill is the appropriate bio-indicator to pollution monitoring.

A. Effect of Fluben diamide on the Liver:

The normal and exposed fishes were sacrificed; the liver was dissected out and was processed for histopathological examination. The normal and exposed liver tissues were microphotographed and are presented in Plate 2 with figures A to E. The normal liver (Fig.
A) of Etroplus maculatus consists of liver lobule as basic unit. The lobule is constructed around a central vein. Radiating out from the central vein are the hepatic cells arranged in the form of hepatic cell plates. They also empty into the hepatic sinusoids. Fluben diamide (Takumin) treated liver showed significant histopathological lesions and the damages were apparent as the exposure prolonged. Different types of pathological lesions could be noticed as in (Plate 2: Fig B to E). Hyperplasia and hypertrophy of hepatic cells were the most notable features in treated fishes. Cells proliferated as well as the size of each cell increased abnormally, which is noted all over the liver tissue.

Normally the structure of the liver of control fish (Plate 2, fig A) showed hexagonal hepatic cells and sinusoidal blood vessels. The fish exposed to sublethal concentration of Fluben diamide initially exhibited vacuolization of cytoplasm, cellular degeneration, hypertrophic and pyknotic nuclei on day 7 (Plate 2 Fig B). However, on further exposure to day 14, certain degree of reorganization in the structure of liver cords was observed. The hepatocytes lose their hexagonal shape, show cellular degradation, with a little degree of cytoplasmic vacuolization, along with degeneration of hepatocytes and degeneration of blood vessels. These changes were observed throughout the tissue (Plate 2 Fig C). At the 21st day of exposure, the liver showed acute inflammation including rupturing of hepatocytes, necrosis, vacuolization of tissue, degeneration of blood vessels, accumulation of pyknotic nuclei and congestion of hepatic tissue were noticed (Plate 2, fig D). After 28 days of treatment, the liver was highly damaged, lost their cytoplasmic density and appeared opaque in many areas, indistinct cell boundaries in many places and pyknotic nuclei were observed. Degeneration of hepatocytes showing distinct vacuoles and necrosis with sinusoidal lesions were the maximum alterations observed (Plate 2, fig E). As the duration increased, severe degradation of the liver cells or hepatocytes and hypertrophy of hepatic nuclei and clumping were evident in many places (Plate 2, fig E).
IV. DISCUSSION

The histological changes in fish is a noteworthy and promising field to understand the extent to which changes in the structural organization are occurring in the organs due to environmental pollution. The histological changes observed in the tissues of E. maculatus indicate that sub lethal concentrations caused moderate to severe alteration in gill and liver, which are important organs performing vital functions like respiration, osmoregulation, detoxification, etc.

The gills, which participate in many important functions in the fish such as respiration, osmoregulation and excretion, remain in close contact with the external environment and are particularly sensitive to changes in the quality of the water are considered the primary target of contaminants (Camargo and Martinez, 2007; Fernandes and Mazon, 2003). The gills of fish exposed to low dose showed curling and clubbing of secondary lamellae. Enlargement of primary lamellae and loss of secondary lamellae were seen at later days of exposure. (Plate 1Fig. B, C, D&E) These pathological changes may be a reaction to toxicants intake or an adaptive response to prevent the entry of the pollutants through the gill surface may be due to increased capillary permeability (Olurin et al., 2006). The present results are in agreement with those observed in other fish species under the influence of different pollutants (Kakuta and Murachi, 1997; Olurin et al., 2006).

The organ most associated with the detoxification and biomarker process is liver and due to its function, position and blood supply, it is also one of the organs most affected by contaminants in the water (Camargo and Martinez, 2007). The liver of the fish exposed to sub lethal dose showed vacuolar degeneration, swelling in the hepatocytes with indistinguishable cellular outline. (Fig. 1 A, B, C) These changes may be attributed to direct toxic effects of pollutants on hepatocytes, since the liver is the site of detoxification of all types of toxins and chemicals. It seems that there is a temporal sequence of the events that starts with vacuolization, swelling and necrosis. Rodrigues and Fanta (1998); Camargo and Martinez, (2007); and Mohamed, (2009) have also reported parallel observations with pesticides in various fishes. Cadmium exposure induces the appearance of granular deposits in the liver, atrophy of the proximal renal tubules, and increases in chloride cell turnover at the gills (Pratap and Wendelar Bonga, 1993). The microphotographs of gill of E. maculatus have been observed and the structure of gills in the fish under controlled conditions, is similar to that of other freshwater cat fish as described by Laurent(1989) whereas the gills of the experimental fish exposed to fluben diamide (7, 14, 21 and 28 days) show active secretion of mucous. More specifically, in the takumin treated gill, the epithelial layer was disrupted. In few regions, disintegration and fusion of primary lamellae were observed, marked hyperplasia of the branchial arch, pilaster cell vacuolization and congestion of blood vessels were well marked. The results are also in agreement with the works of Kishor Haloi et al.(2013) who have reported for Boleophthalmus dumeric exposed to sublethal concentration of cadmium.

In the present study vacuolization, space formation and resulting haemorrhage, hypertrophy of hepatocytes, clumping, extensive necrosis and pyknotic nuclei were observed in takumin treated liver. These types of histological alterations were also noticed in the liver tissue of Labeo rohita exposed to tannery effluent (Rao, et al, 2005). Conclusively, pesticides are stored in different sites in animals depending on the pesticides and on the animal species. To check the continual introduction of these pesticides into the food chain, a more cautious application of insecticides and pesticides should be employed and effluents from industries must be treated before disposal.

It was noticed that the action of the pesticides in sublethal levels did not affect the liver as a whole, but focally. Therefore, a short exposure and a sublethal level of pesticides may allow a recovery. But as happens in other species and under the action of varied pollutants, one can assume that if the action is repeated or stronger, these effects may cause some chronic alterations in the case of a particular pesticide contamination. Liver changes affect directly the metabolism of fish therefore diminishing its life fitness. One can conclude that, with weakened health they may be easily predated, will have lower reproductive capacity and might lose in competition for space or food. Therefore, from an ecological point of view, there might be a change in the population and in the food chains.

Liver being the main metabolic factory of the body, serves several very basic functions such as metabolism, storage, and the secretion of bile. Liver accumulates more toxicants than other organs of body. Liver is the organ, which metabolises the toxicants and excrete it out. Since metabolism of proteins, fats and carbohydrates and detoxification of endogenous waste products and drugs take place in liver, it is more liable to injury from toxicants. Considering the importance of liver, several studies were undertaken to explore the histopathological changes in liver, cellular level complexities and reasons for mortality (Hemnai and Kaldas, 1994; Brock, 1998; Vurk and Sharma, 1999; Sanjoy and Rita, 2012; Abraham and Tresa, 2002). Due to their non-migratory nature, E. maculatus are prone to all sources of pollutants added to the aquatic environment of Kuttanad. The results of the present study indicate that the pesticides such as Fluben diamide also cause far reaching consequences in the aquatic system. Even the sublethal concentration of the Fluben diamide is enough to elicit significant changes in the histology of fishes like Euproplus. The results further suggest that even smaller concentrations of any toxicant in the environment can induce major histological changes and more care and vigil is needed before dumping pesticides or organic/inorganic manure in to agricultural fields or environment. Long term exposure of organisms to pesticides means a continuous health hazard for the population. So, human population also, is at high risk by consuming these toxicated fishes. This implies that one should take the necessary precaution in the application of pesticides to protect the life of fish and other aquatic fauna. Furthermore, the present study also adds to the concept that histopathological studies are one of the effective tools for ecotoxicology and risk assessments.
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REFERENCES