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RESEARCH ARTICLE

IMPACT OF LEAD NITRATE ON THE GILLS OF TIGER SHRIMP, *PENAEUS MONODON* EXPOSED TO SUBLETHAL CONCENTRATIONS LEADING TO HISTOLOGICAL CHANGES

[1*]Palani Kumar, S., [2]Sharadhamma, A.S., [3]Swamydoss Daniel, G., and M. Aneez Mohamed[4]

[1]Department of Zoology, A.V.V.M., Poondi Pushpam College, Thanjavur, Tamilnadu, India
[2]Assistant Surgeon, Government primary Health Centre, Thanjavur, Tamilnadu, India
[3]Controller of Examinations, PRIST University, Vallam, Thanjavur, Tamilnadu, India
[4]Department of Zoology, Jamal Mohamed College (Autonomous), Tiruchirappalli, Tamilnadu, India

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ABSTRACT

In the present study the tiger shrimp, *Penaeus monodon* were exposed to three sublethal concentrations of Lead nitrate (1.66, 3.33 and 6.60 mg/L) for a period of 24 hrs,48 hrs,72 hrs,96 hrs respectively. The Gills of the shrimps were then dissected out and processed for light microscopy studies. Exposed shrimps were found to result in several alterations in the histoarchitecture of Gill. The alterations included: necrosis of the epithelial cells of the Gill, hemocytic infiltration in the interlamellar spaces, were observed in the Gills. The results obtained suggest that the Gills of shrimps exposed to sublethal concentrations of Lead nitrate were structurally altered. Such alterations-could affect vital physiological functions, such as absorption, storage and secretion of the Gills, which in turn could ultimately affect the survival and growth of *Penaeus monodon.*

INTRODUCTION

Metal accumulation in the environment continuously increases owing to the anthropogenic activities and they tend to concentrate in all the aquatic matrices. The low-dose heavy metal exposure to aquatic organisms may result in various manifestations of biochemical, physiological, and histological alterations in primary tissues (Hinton *et al.,* 1973; Ghate and Mulherkar 1979; White and Rainbow 1986; Kaliamurthy *et al.,*1994; Yamuna *et al.,* 1996). Heavy metals occur naturally in the environment and are found in varying levels in all ground and surface waters (Martin and Coughtrey, 1982). According to Mason (1991), heavy metal pollution is one of the five major types of toxic pollutants commonly present in surface waters. The important environmental pollutants are those that tend to accumulate in organisms, those that are persistent because of their chemical stability or poor biodegradability, and those that are readily soluble and therefore environmentally mobile (Hellawell, 1986; Sanders, 1997). Histology, the microscopic study of tissue, provides essential knowledge of living cells as the basic building blocks of all living organisms. It examines the structural organisation of these cells in different tissue types allowing better understanding of morphological aspects and physiological processes of different organs. According to Short and Meyers (2001), histology is an important field regarding fish health that can often detect subtle conditions or early signs of disease not easily recognized on gross examination. Results from a histological assessment, can provide better insight into the environmental and/or physiological demands presented to fish in their natural environment (Short and Meyers, 2001). Heavy metals including lead are found in various tissues of fish and shrimps (Vazquez *et al.,* 2001).The ultrastructural alterations are suggestive of the operation of compensatory mechanisms within the test prawns to enable it to tolerate Hg toxicity. However, these alterations would have an impact on the cellular integrity of the gills and hepatopancreas and such alterations can be taken as 'biomarkers' for assessing Hg pollution in the aquatic environmen (Yamuna *et al.,* 2009). Therefore, it is likely that exposure to a noxious chemical, such as a Lead nitrate, would be reflected in alterations in the structure of the tubules and epithelial cells. Several such structural alterations were noted in the hepatopancreatic tubules of test shrimps, which had been exposed to lead nitrate in the present study. The aim of the present study is to assess the histological changes in the tiger shrimp, *Penaeus monodon* exposed to sublethal concentrations of Lead nitrate. Which is an economically important species cultured in India. The microscopic study of the tissue provides essential knowledge of living cells as the basic building blocks of all living

Corresponding author: drspkumar71@yahoo.in

organisms. It examines the structural organization of the cells in different tissue types allowing better understanding of morphological aspects and physiological process of different organs.

MATERIAL AND METHODS

Experimental animals

The shrimps were collected from culture pond at Velankanni, Latitude: 10.6833, Longitude: 79.8333, Lat (DMS): 10° 40' 60N, Long (DMS): 79° 49' 60E, Time zone (est) of Nagapattinam District, South India. The shrimps were acclimatized to laboratory condition by using in-door fiber tanks, each with 1.5 m in diameter and 1 m in height, containing 0.80 m water with adequate aeration, and 20% of water volume changed daily. The shrimps were acclimated under the light–dark cycle of 12:12 for two weeks before the experiment. The water temperature was maintained at 27–28 °C. Salinity, 40 ppt; total hardness, 255.0 mg/l; pH, 8.2; nitrate, 1.6 mg/l; chloride, 27.0 mg/l; ammonia, 0.058 mg/l; dissolved oxygen, 6.7 mg/l; BOD, 5.8 mg/l; COD, 14.7 mg/l; and total solid, 1.7 g/l). Commercial shrimp feed was provided daily at 3% of the body weight.

Tissue preparation for histological observation

The five to eight posterior gills were dissected and immediately fixed in Bouin's fixative for 48 h. The preserved tissues were processed by a routine histological method (Gurr 1962), dehydrated in an alcohol series, cleared in xylene, infiltrated with liquid paraffin at 58 °C, and finally embedded in paraffin blocks. The blocks were trimmed and sectioned at 5–8 µm thick cut on a rotary microtome (Weswox MT Chenai, India), were stained with Harris' Hematoxylin and counter-stained with Eosin (H&E stain). Then the slides mounted with DPX and observation under a light microscope (Woods and Ellis, 1994).

RESULTS

Control

Inter lamellar space normal, No Haemocytes infiltration, Gill tips are normal Cells are normal, Pillar cells are normal, Haemocytes not clear , No cell necrosisiof gill lamellae and cells ,Epithelial cells are normal L, Lamellar epithelium are normal. The gills are well developed nourished and the primary gill filaments are attached to the branchial arch and each supported by an independent gill ray.A number of secondary lamellae are seen attached on both sides of primary filament any they are the main part of gaseous exchange The C.S of gill shows secondary lamellae comes out as fingershaped from the supportive rays .Histologically, the gill shows clearly the occurrence of connective tissues,core cartilage, glandular epithelial cells and filamentous cells excluding arterioles and veinules. The shape of the filament cells are spherical or oblong with a nuclei.The cytoplasm stains more with eosin than cuboidal cells.The cuboidal cells are glandular and responsible for secretion of mucous but the filament cells are essential for respiratory functions (Plate A).

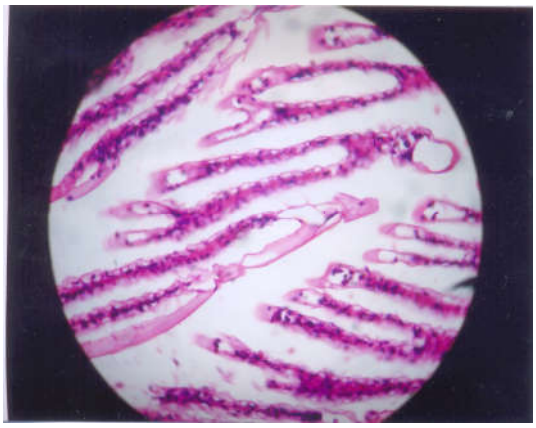

Plate A. Penaeus monodon – Gill (Control)

Plate B. Damage in the basal attachment area (Branchial arch) Cells were damaged and shows sign of deformities SLC-I

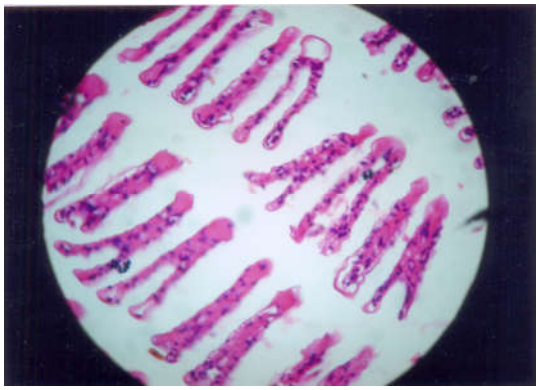

Plate C. Lifting of lamellar epithelium Inter lamellar space seen nucleated cells seen SLC-II

Sublethal conc. I (1.66 mg/L)

The impact of Lead nitrate shows mild structural changes and damage on the basal attachment of gill. Deformities in the (Branchial arch) basal gill attachment area .The cells show signs of damage and deformities. (Plate B)

Sublethal Conc. II (3.33 mg/L)

In this concentration the inter lamellar space seen, Lifting of Lamellar epithelium. Necrosis of gill lamellae and cells. Abnormal gill cells and gill tips were damaged. (Plate C)

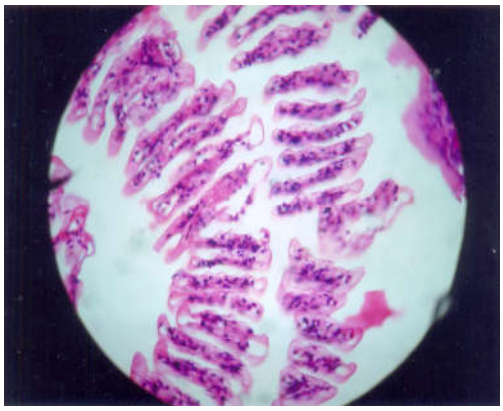

Plate D. Gill tip damage and deformities in cellular arrangements, necrosis

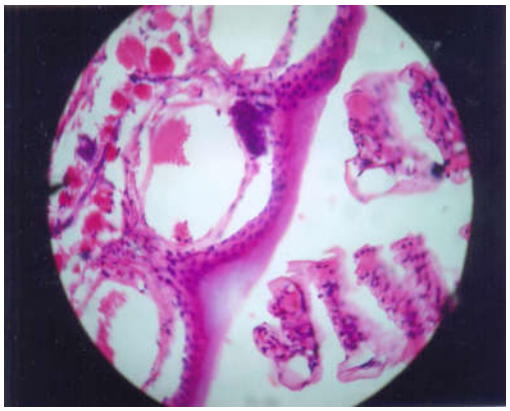

Plate E. Lacuna seen with abnormal gill cell tip damages – SLC-III

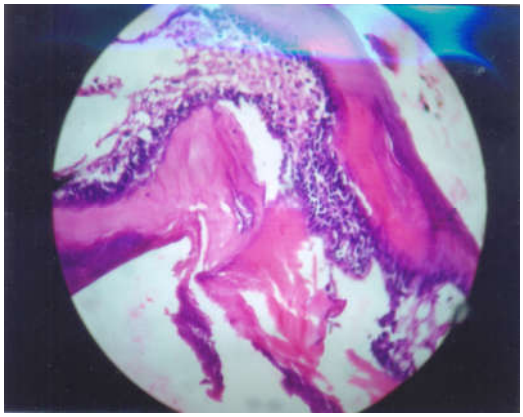

Plate F. Deformed cells showing damage in the gill basal attachment Lead nitrate treated SLC-III

Sublethal Conc. III (6.60 mg/L)

Deformities in the (Branchial arch)basal gill attachment area. The cells show signs of damage and deformities.Abnormal gill cells and gill tips were damaged. Vaculation (Lacunae) is seen. Pillar cells damaged. Proliferation of epithelium. Lifting of Lamellar epithelium. Necrosis and erosion of the filaments are seen in the distal ends .The lead nitrate has impact over the gill cells. Primary gill filaments are attached to the .branchial arch damaged. Extensive damage of gill structure and cellular alterations are observed. (Plate D)

DISCUSSION

Following exposure the gills exhibited rapid alterations that include detachment and lifting of the epithelial linings from the surfaces of the gill filament (primary, PL) and respiratory (secondary, SL) lamellae. This leads to extensive haemorrhage from the gills. Thus the quantity of blood flowing across the gills decreased substantially. Simultaneously, uncontrolled regeneration of the PL and SL occured, leading to extensive hyperplasia of the epithelial cells lining the PL, and SL. Consequently, the gill filaments appeared as a cylindrical solid mass of cells with very little or almost no free surface left on the SL for gaseous exchange. The goblet mucous cells also exhibited periodic fluctuations in their density and staining behaviour. The chloride cells showed periodic fluctuation in their number at different stages of exposure. The density of the chloride cells is inversely proportional to the thickness of the epithelial lining of the PL and SL. Due to prolonged exposure, the neighbouring SL fused together and the entire gills appeared as solid mass of undifferentiated cells. Subsequently, the ladder-like arrangement of the pillar cells-blood capillaries of the gills also collapsed, causing asphyxiation and the death of the fish.Similarities observed and coincides with Ram Sanehi Parashar, and Tarun Kumar Banerjee, (2002).

CONCLUSION

Thus, the changes observed in the shrimps exposed to 1.66,3.33,6.60 mg/L lead nitrate in the present study were more likely to have represented a progressive loss of basic biological functions of the Gills and it ultimately affect the survival and growth of *Penaeus monodon.*

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