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# EFFECT OF MERCURIC CHLORIDE ON GILL STRUCTURE OF A FRESHWATER FEMALE CRAB, BARYTELPHUSA CUNICULARIS (Westwood)

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**Abstract**

Fresh water crabs are economically important and an alternative source of significant human dietary constituents. The experimental crabs treated with lethal concentrations (1.04, 0.84, 0.63 and 0.45 ppm) of mercuric chloride showed many histological changes during 1 to 4 d of exposure. The gills showed vacuolization in the gill stem, gill lamellae ruptured, connective tissue cells in the stem damaged, destructed and congestion of haemocytes in the gill lamellae are observed. The present work on histological observation was carried out to know lesions in gills that had resulted from lethal exposure of the freshwater female crab, *Barytelphusa cunicularis* (Westwood) to mercuric chloride.

Key words: Mercuric chloride, histology, gills, *Barytelphusa cunicularis*.

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## INTRODUCTION

Histological studies have a way for understanding the pathological conditions of the animal by helping in diagnosing the abnormalities or damages of the tissues exposed to toxic stress of heavy metals (Sprague, 1971; Andhale, *et al.,* 2011, Maryam, *et al.,* 2013). Histological changes not only give an early indication of pollution hazard, but also provide useful data on nature and degree of damage to cells and tissues (Shaikh, 2010).

The gills of aquatic organism represent primary target of disturbance by pollutants as they are in direct contact with the external medium to perform gaseous exchanges and ionic regulations. This organ has linking sites to promote its regular functions, and these sites link to toxic substances with differentiated charges, triggering, mechanical responses and toxic effects to the organism (Jadhav, *et al.*, 2007, Li, *et al.*, 2007).

Kale (2002) observed the histological changes in gills of the crab, *Barytelphusa cunicularis* due to cadmium toxicity and observed ruptured gill lamellae. Histotological changes in tissues are biomarkers of effect and exposure that integrate responses to contaminants at the cellular level (Wu, *et al.,* 2008).

Vijayalaxmi and Tilak (1996) while working on *Labeo rohita* found pathological lesions in gill exposed to pesticides. Vernberg and Vernberg (1972) studied the changes in the gill tissue of crab after exposure to sublethal concentration of mercury. Saksena and Pandey (1993) also have observed toxic effect of copper on the histological effect on gills of a freshwater fish, *Labeo rohita.* In 1990, Victor *et al.,* observed the changes in the gill tissue of freshwater prawns, *Macrobrachium idae* exposed to mercury.

A review of literature shows that there are more study is require to observed the histological changes caused by heavy metals in gills of *Barytelphusa cunicularis.* Hence, the present study was undertaken to study the effects of mercuric chloride on histological changes in the gills of *Barytelphusa cunicularis*.

## MATERIALS AND METHODS

*Collection of crab:* The freshwater crabs, *Barytelphusa cunicularis* were collected from the outskirt of Aurangabad City. They were acclimatized to laboratory conditions under normal day/night [11 L : 13

D] illumination at 27 ± 1º C for one week in plastic troughs (18" diameter) containing sufficient tap water so that crabs are submerged. Before experimentation intermoult female crabs (Stage $C_3$; Diwan, 1973) of approximately equal carapace width (45-50 mm) and body weight (50 to 55 g) were sorted. For histological study crabs were split into 2 groups (Control, $HgCl_2$ treated), 5 crabs in each group and maintained under laboratory condition.

After exposure period surviving crabs were sacrificed and the gills were quickly excised and utilized for histological studies from both the control and experimental crabs.

*Histological studies:* The excised tissues were fixed in aqueous Bouins fluid. After fixation for 24 h the tissues were further processed to study histological details as per procedure of Bancroft and Stevens (1982). In brief the tissues were dehydrated through 30 - 100 % different alcohol grades and cleared in xylene. Cold and hot impregnations were followed by embedding the tissue in paraffin wax (M.P. 58-60 ° C). Serial sections were cut at 7 µm serial using rotary microtome. The section of gills was stained using Harris Haematoxylin and Eosin-Y as counter stain (Bancroft and Stevens, 1982). Damage to the tissues of treated crabs is recorded by comparing the data obtained from control.

## RESULTS

The histological study has been carried out to know lesions in gills that had resulted from lethal exposure of the freshwater female crab, *Barytelphusa cunicularis* to mercuric chloride. In freshwater crab gills are paired and have crescent shaped structure and located laterally on either side of the hepatopancreas and alimentary canal within the gill chamber in the antero-ventral region of thoracic region. Gills are normally deep brown in colour.

**Histological structure of the gills in control crab.**

The gills of *Barytelphusa cunicularis* are phyllobrachiate type consisting of central stem (axis, raphe) that bears serially the paired plate or leaf like lamellae, which are actually flattened sacs. The central axis has afferent and efferent haemal channels on each end. Three types of cells are found: (1) *Connective tissue cells* are located in the gill stem; (2) *Branched arthrocytes* are located in the stem and proximal lamellae; (3) *Lamellar cells* are located in the epithelium. Each gill plate has outer cuticular layer enclosing a single layer of gill epithelial cells. The gill epithelium stained deep pink in haematoxylin and eosin. The central axis contained large amount of darkly stained haemocytes. The central axis also had few nephrocytes. The nephrocytes are vacuolated large cells that have some amount of pale brown material [Fig. 1-A].

*Histological changes in the gills of experimental crab*

The experimental crabs treated with lethal concentration of mercuric chloride showed many histological changes during 1 to 4 d of exposure. In the experimental crab gills showed vacuolization in the gill stem, gill lamellae ruptured, connective tissue cells in the stem damaged, destructed and congestion of haemocytes in the gill lamellae are observed. Pillar cells are damaged. The thin connective fluidy band present in between the two gill lamellae is found ruptured (Fig. 1B).

## DISCUSSION

The experimental crabs exposed to lethal concentrations of mercuric chloride exhibit histological changes in the gills due to the accumulation of mercuric chloride within the organism body at lethal and levels lead to histological lesion in the body crab, *Barytelphusa cunicularis.*

Histopathological studies are also useful in evaluating the pollution potential of heavy metal pesticides, since trace amount of these chemicals which do not bring animal mortality over a given period, were capable of producing considerable organ damage (Kumar and Pant, 1984; Jaykumar, 2002). Despite much information available on the histological changes caused by heavy metal pesticides, the mode of action on the vital organs is still not fully understood.

The difference between the control and the experimental tissues were studied critically. The study of micro-anatomy (histology) of the specific tissue constitutes an important diagnostic tool to observe the histological effects caused by a pollutant. The histological changes may be the manifestation of sick tissue (Kamble and Potdar, 2010).

Gills of the crab, *Barytelphusa cunicularis* were of phyllobrachiate type with a central axis and gill lamellae arranged in two rows on either side (Fig.-1 A and B). The structure of the gills observed in

the present study was well in accordance with earlier reports on different crabs (Joshi, 2006; Jadhav, *et al.*, 2007).

In experimental crab the histological sections of gills showed vacuolization in the gill stem, gill lamellae ruptured, connective tissue cells in the stem damaged, destructed and congestion of haemocytes in the gill lamellae are observed. Pillar cells are damaged. The thin connective fluidy band present in between the two gill lamellae is found ruptured (Fig. 1-B). Li *et al.*, (2007) observed structural changes including the swelling and fusion of the lamellae; abnormal gill tips; and necrotic lamellae in gills of a giant freshwater prawn, *Macrobrachium rosenbergii* exposed to waterborne copper. Kurian and Radhakrishnan (2002) observed such changes in the gills of the field crab, *Paratelphusa hydrodromus* on exposure to nickel.

The histological techniques are the promising area of research in aquatic toxicology as it gives the real picture of the effects imposed and the involvement of the xenobiotics in either disturbing or destroying the vital organs of living organisms. Many researchers have reported the degenerative changes in selected tissues of the animals in response to pollution by various toxicants (Shanmugam *et al.*, 2000; Suresh, 2001; Kale, 2002; Reddy, 2005; Tilak, *et al.*, 2005; Andhale, *et al.*, 2011,Mukke, 2012).

## CONCLUSION

The histology is a most common tool for determining the deleterious effects of toxic substances on the treated animals. In the present investigation an attempt has been made to evaluate the intensity of the damage done to gills of crab subjected to its lethal concentrations of mercuric chloride.

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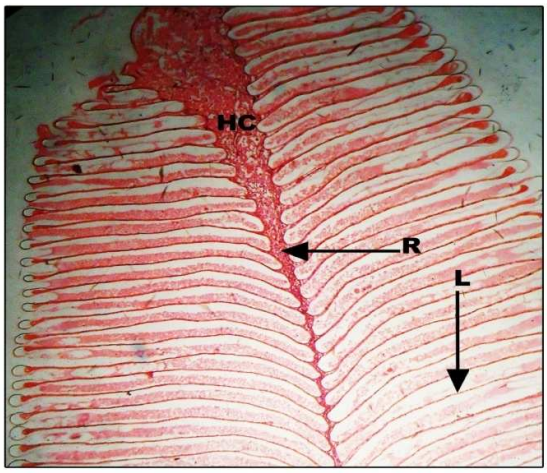
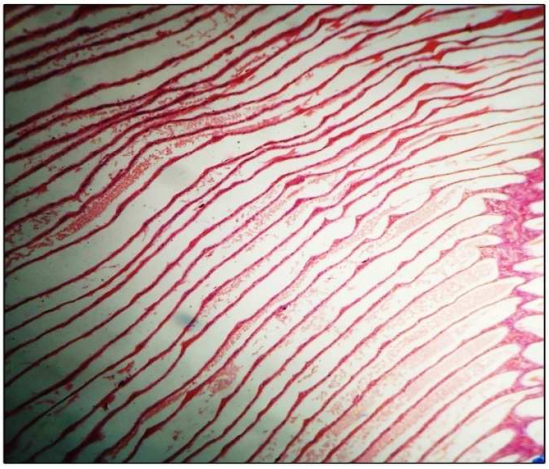



a. Control crab x 400: Distinct haemotocytes, gill stem, gill lamella withthin connective fluidy bands are observed;



b. Experimental crab x 200: Vacuolization of gill stem, fusion and ruptured gill lamella, destructed and congestion of haemocytes, thin connective fluidy bands are ruptured.

**Fig. I: T.S. of gills of crab, *Barytelphusa cunicularis*.**

HC: Haemocyte, L: Lamella and R: Raphe (Axis). Stain H and E.

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