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Mercury Induced Changes in Hepatopancreas And Gonads Of Fresh Water Bivalve *Lamellidens Marginalis*

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ABSTRACT
Histological studies would help in evaluating the extent of damage caused to the tissues under stress. The bivalve Lamellidens marginalis were exposed to LC_0 and LC_{50} values of 96 hrs with concentrations of 0.120 and 0.687 ppm for $HgCl_2$ metal salts respectively. The results showed that the mercury caused deteriorative changes in all concentrations. During different hour exposure in higher concentrations of mercury metal the tissues showed swelling of the tubules, which was distinct from the connective tissue. The number of amoebocites in the connective tissue decreased considerably. However, during acute exposed period in lower concentration of metal the tissues resulted the tubules completely lost their original shape probably due to dissolution of the basement membrane.

KEY WORDS: *L. marginalis, mercury, acute toxicity, gonads*

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I. INTRODUCTION

Bioaccumulation of heavy metals by bivalve molluscs such as fresh water bivalves might have implications on human exposure in India due to consumption as food. Year around monitoring of these shellfishes may provide adequate information on local discharges and seasonal variation in concentration of contaminants including heavy metals. High levels of heavy metals in different molluscs have been reported from different parts of the world (Wang and Ke 2002 and Suryawanshi 2002). Considerable work has been done on toxicity of heavy metals to economically important bivalves with reference to changes in hepatopancreatic structure due to heavy metals (Sivaramakrishna and Radhakrishnaiah 2000, Domouhtsidou. and Dimitriadis 2000 and Liu *et al*., 2010). Histology is an indispensable and powerful technique in establishing routine toxicology studies performed for the purpose of assessment of edible resources to human beings. Its value lies not only in the sensitivity in terms of toxic levels but particularly in the disclosure of large organs and mechanisms of action. To assess its usefulness in toxicology studies with fish and shellfish, several investigators have performed experiments using various environmental contaminants (Cajaraville et al., 1990, Wester 1991, Usheva *et al*., 2006 and Raftopoulou and Dimitriadis 2011).The hepatopancreas is a main organ of storage of metabolic reserves, which provides a source of energy during the periods of physiological stress in addition to its role in the digestion of food. The impairment of the cell architecture of hepatopancreas due to heavy metal stress definitely reflects in its metabolic disorder. Marrigomez *et al*., (1990) reported histopathological changes in the digestive gland–gonad complex of the marine prosobranch, *Littorinalittorina* exposed to cadmium. The cellular responses to mercury and mercury in the digestive cells, cilia and digestive tubules of *Perna viridis* and *Diplodonchilensis* were studied Sebastián *et al*., (2014).The knowledge of reproductive cycle is necessary for predicting annual recruitment, interpreting growth, mortality and survival data and also in culture of species. In bivalve mollusks the reproduction has been studied by many researchers and much of the work has been reviewed exclusively on freshwater species and much of the literature on reproduction in bivalve mollusks is concerned with the gonad development and reports on breeding periods (Wagner and Boman 2004 and Wepener *et al*., 2008). Several environmental factors have been shown to control the reproductive cycle. Gametogenesis begins shortly after growth and maturation of the gonad (Ortiz-Zarragoitia, and Cajaraville 2006). For freshwater species the studies comprise by dividing the gonad into a number of stages based on microscopic examination of histological sections in different parts of the year, on *Lamellidens marginalis* Patil, and Mane (2000), on *P. corrugata* Waykar and Shinde (2013). It is in this view that the present study was done on the histological changes of depot tissue, hepatopancreas and gonads of the mussels *L. marginalis* exposed to mercury under laboratory conditions.

II. MATERIALS AND METHODS

During study the mussels *L. marginalis* were collected from Nagapur dam and soon after the collection they were brought to the laboratory and the shells were brushed to clean the fouling biomass and mud. The acute toxicity tests were performed on the bivalves using lethal concentrations LC^0 and LC^{50} values of mercury chloride. The control group in normal was run simultaneously during experiment. The animals belonging to control and experimental were sacrificed separately to obtain hepatopancreas and gonad and these were fixed in Bouin'sHollande fixative for 48 hours. After fixation the tissues were then washed in distilled water and dehydrated in ethyl alcohol in different grades, cleared in xylene, embedded in tissue mat (at 58-60 ^{o}c melting point) and these were then sectioned at 5-6µm thickness on rotary microtome. These sections were stained with Mallory triple stain and mounted in DPX. All the observations and microphotography were made under research microscope.

III. RESULTS

The changes in hepatic tubules and its cellular structure due to metal toxicity ofbivalves along with control are shown in (figures 1-2). In control group of bivalves the hepatopancreas consists of ducts and digestive tubules grouped in the form of bundles indistinctly separated and connected interlobular connective tissues of collagenous fibers. Each tubule is bounded by thin muscle fibers, which form the basement membrane. Each digestive tubule consists of digestive cells, vacuolated and acidophilic and pyramidal type and basophilic type cells. When the bivalves exposed to metal concentrations of mercury the residual bodies or fragmentation spherules were comparatively more in the lumen in lower concentration than in higher concentration. Furter the results showed that the mercury caused deteriorative changes in all concentrations comparatively metal concentrations. During different hour exposure period in higher concentrations of mercury the tissue showed swelling of the tubules, which was distinct from the connective tissue. The number of amoebocites in the connective tissue decreased considerably. The observations of the gonads (fig-2) showed the lumen was expanded in the metal accumulation period of LC^0 as well as acute exposure period. Further the female ovaries many vitellogenic oocytes were found and being released in a few follicles degenerating oocytes were also seen in LC^0 exposed bivalves.

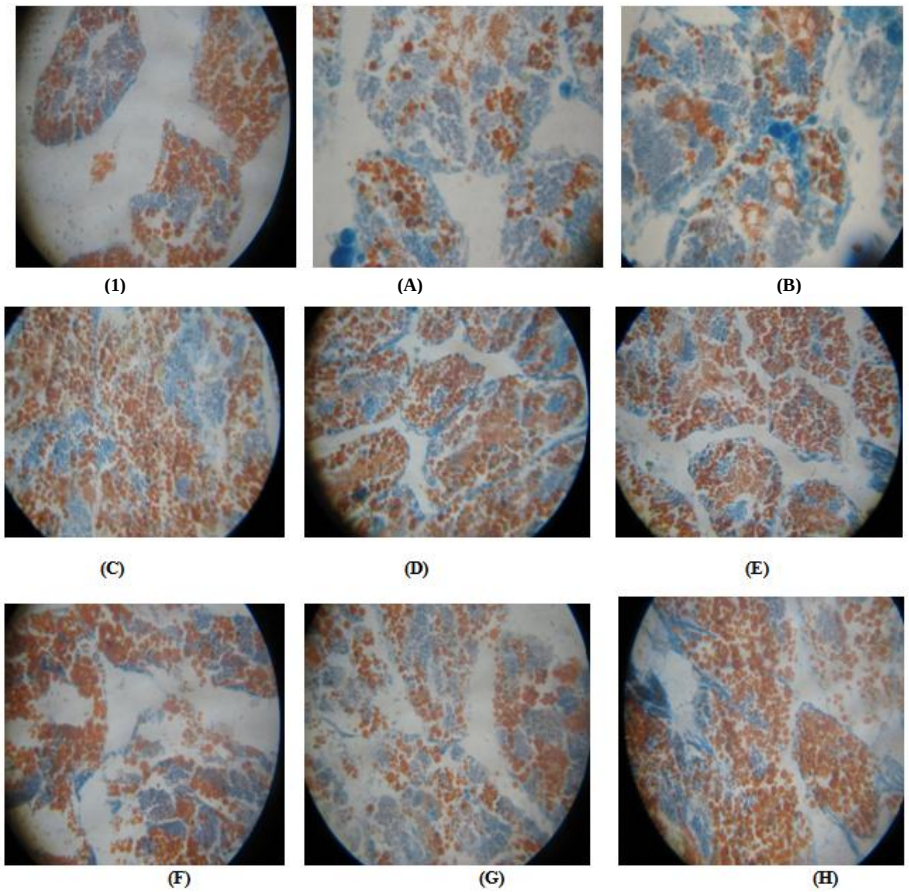

Fig. 1: Histological changes in hepatopancreas of *L. marginalis* exposed to mercuric chloride for 24, 48, 72 and96hrs.1=Control, A=LC_0, B=LC_{50} for 24 hrs, C=LC_0, D=LC_{50} for 48 hrs, E=LC_0, F=LC_{50} for 72 hrs, G=LC_0, H=LC_{50} for 96hrs,

During acute exposure period in lower and higher concentrations of mercury metals the follicle wall ruptured at places and follicular content showed necrotic condition. This was more pronounced in LC^{50} group than the LC^{0} group follicle remained compact. Whereas during LC^{0} exposure period showed many previtellogenic and vitellogenic oocytes were formed. Prominent vacuole appeared in their nuclei and lipid globules and nutritive cells decreased much in quantity.

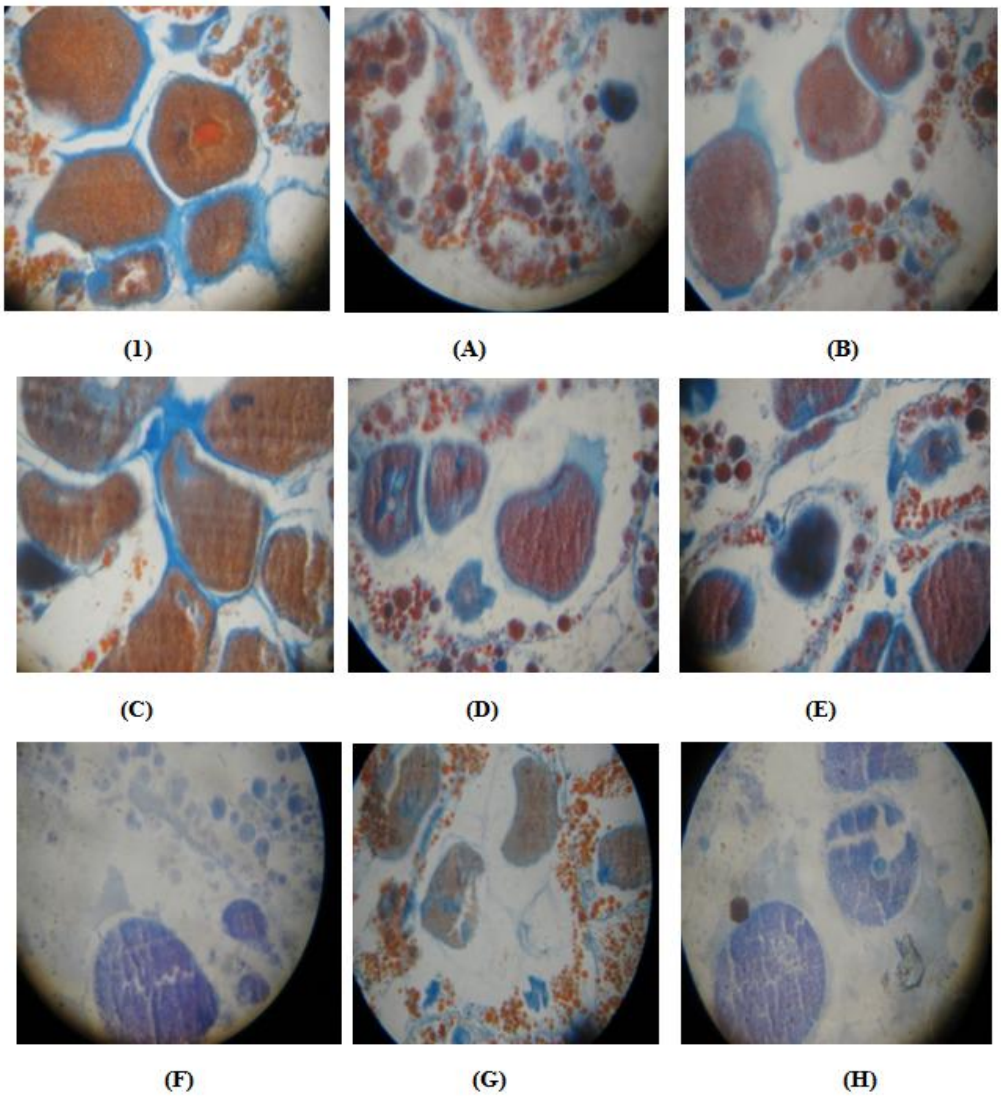

Fig 2: Histological changes in gonads of *L. margianalis*exposed to mercuric chloride for 24, 48, 72 and 96 hrs. 1= Control, A=LC_0, B=LC_{50} for 24 hrs, C=LC_0, D=LC_{50} for 48 hrs, E=LC_0, F=LC_{50} for 72 hrs, G=LC_0, H=LC_{50} for 96 hrs,

IV. DISCUSSION

The freshwater environment, where concentrations of essential trace metals vary from limiting to toxic levels, particularly challenges an organism's ability to maintain appropriate reservoirs of essential metals. This problem is often compounded by the presence of significant concentrations of metals that can compete with uptake sites and intracellular binding sites. Such competition reduces the concentration of metals that are effectively available to the organism. The destruction of cellular structure of hepatopancreas due to pollutants definitely reflects in the metabolic activity of it. However, in *M. edulis* no significant reduction in lysosomal integrity was evident in the group exposed to the lowest mercury concentration; however, significant reduction in lysosomal latency was observed in the group exposed to highest concentration of mercury as observed by (Kulkarni 1993 and Sheir *et al*., 2010). Shaikh *et al*., (2012) observed the heavy metal like cadmium was effective to *L. marginalis* to cause cellular destruction of hepatic lobules in winter season. Similarly in present study showed *L. marginalis* vacuolization and increase in intracellular space within the tubule was observed than in the control group. The digestive and secretary cells were both affected at cellular levels at different

intensities the destruction was more pronounced in higher concentration of mercury and mercury than lower concentration, which might be due to higher intensity of metals, affected in higher concentration. Moore and Ramamoorthy (1984) stated that pathological reactions of the lysosomal system in hepatopancreatic cells of bivalve mollusks have proven to be sensitive bioindicators of pollutant effect. Further, they studied the effects of several cell-to-cell signaling compounds on the lysosomes of the hepatopancreatic digestive cells.The freshwater environment, where concentrations of essential trace metals vary from limiting to toxic levels, particularly challenges an organism's ability to maintain appropriate reservoirs of essential metals. Moreover, it encompasses a number of events, including fertilization, setting and organogenesis that are particularly sensitive to stressors. Perturbations in any of these processes can result in arrested or abnormal development. Cajaraville et al., (1990) while studying on the mussel *M. galloprovincialis* exposed to crude oil and commercial lubricant oil indicated that these changes in the lysosomal compartment appear to be associated to the process of gametes release or spawning. In the present study abnormalities developed in the gonadal cells, leading to the appearance of enhanced growth of these cells of *L. margianlis* could be also due to the underlying mechanisms in the functional changes brought about by the different duration of exposure period. Organisms have evolved various strategies that appear to lower the effect of environmental changes; these include avoidance responses, repair or stabilization mechanisms and synthesis of detoxification enzymes or binding ligands. Presumably these strategies have evolved with a minimal cost in energy or rate limiting nutrients, although all of these processes require energy or nutrients that are diverted from other uses. Mercury appeared to cause more extensive mitochondrial damage than did treatment with mercury. In the present study, it is possible that penetration of heavy metals in the gonad tissue of *L. marginalis* could have resulted in the changes in lysosomal compartment of vitellogenic oocytes, particularly in higher concentration and acute exposed bivalves due to which the mature gametes instead of being spawned out showed degenerative changes. Based on these findings it can be presumed under present study that vitellogenic oocytes formed due to metal stress are retained on metaphase I^{st} and no short-lived proteins are synthesized because of the formation of metalloproteins. In addition, it is possible that the total or partial destruction on the synthesis of vitellin- like protein from the haemolymph and related ovarian proteins could have caused the little large sized oocytes to undergo lysis after metal stress. It has been reported that the achievement of gametogenesis in adult bivalve mollusks, *M. edulis* during reproductive cycle depends upon the action of different neuroendocrine factors, a mitotic factor allowing the multiplication of oogonia and spermatogonia and a vitellogenic factor acting on vitellogenesis Wang and Ke (2002). To deal with these challenges, freshwater organisms have evolved a number of adaptive strategies that are collectively referred to as metal metabolism.

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