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Research Article

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Cadmium induced histopathological alterations in female gonad of freshwater bivalve mollusks, *Lamellidens marginalis* during summer season

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Abstract: The study was aimed to investigate the effect of cadmium on the gonad in female fresh water bivalve mollusks, *Lamellidens marginalis*. The experiment was carried out on the bivalves collected from Kutluq Lake, Daultabad, district Aurangabad of Maharashtra, India from January 2018 to December 2018. The histolopathological findings include partial disruption of ovarian follicle, vacuolation of germinal cells cytoplas m and damaged inter follicular connective tissue. The cytomorphological structure of ovarian follicles got deformed and elongated, losing their typical configuration. Necrosis and fibrosis in the connective tissue and damage to yolk vesicles of maturing sites were observed.

Keywords: Cadmium, Female gonad, Histopathology, *Lamellidens marginalis*.

INTRODUCTION

Histopathology is an indispensable and powerful technique in establishing routine toxicology studies performed for the purpose of risk assessment of living resources useful exposing the tissue contents and mechanisms of action at cellular level. To assess its usefulness in toxicology studies with shellfishes, several investigators have performed experiments using various environmental contaminants (Wester and Canton, 1991).

Many species of bivalve mollusks abundantly found in Indian waters can sustain regular & very productive Fisheries in India, particularly in Maharashtra state. Several species of commercial, important and edible bivalves like clams, oysters mussels etc. are found along the coastal areas whereas other bivalve shell fishes like mussels and clams are found both in lotic and lentic freshwater bodies. Both marine and freshwater bivalve shellfishes play an important role as bio-indicators to detect various environmental fluctuations (Dholakia 2013 and Shinde Nitin 2017).

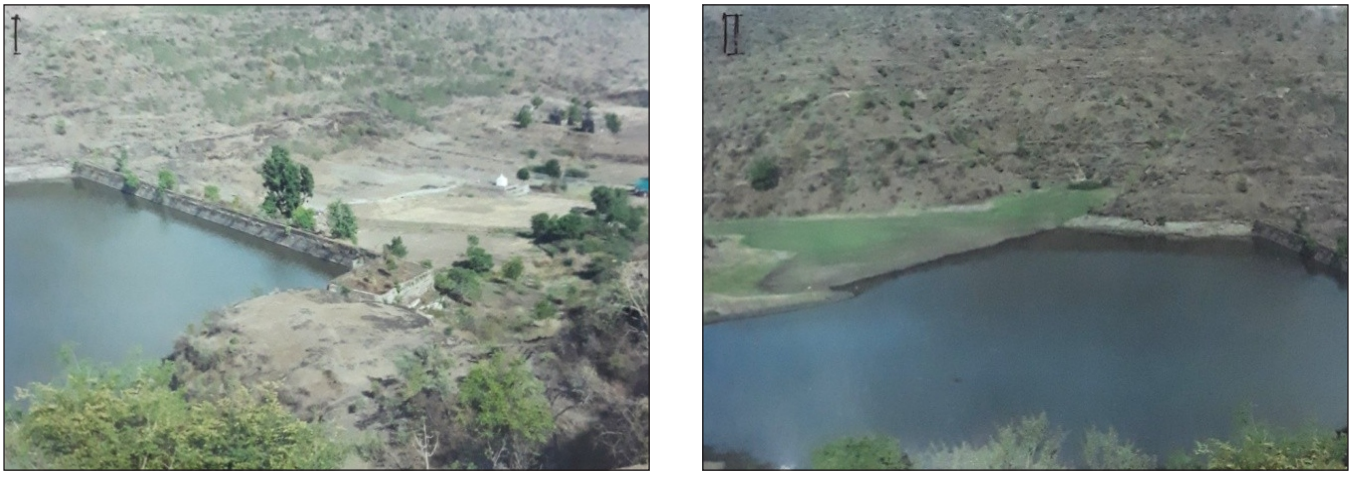
In order to understand a pattern of damage caused by particular chemical to the tissue, it is essential to have an insight into the histological analysis of the tissues. Histopathology, deal with the study of pathological changes of the microscopic structure of the body tissue. Any peculiar type of alteration of cells may indicate the presence of the disease or the effect of toxic substances. Every organism has capacity to tolerate suboptimal stress conditions. The maximum tolerance is at the extreme stress condition which exhibits physiologically defined limitations, reflected predominantly in the structural architecture of various tissues of the animal. Traces of toxicants introduced in the body can be neutralized by immune system, but when high amount of toxicant enters, it affects the structure and function of different organs in the body of animal. Thus, histopathology is an extremely useful tool for assessing effects of toxicants at individual level. Their uncontrolled use may cause profound effects and a long term environmental impact on natural aquatic environments (Bellas *et. al*., 2004). Pesticides may impair the functioning of organisms in aquatic environment even at low concentration.

Histopathological abnormalities caused due to toxicity of pesticides have been reported earlier by many investigators (Muley and Mane, 1990; Jonnalagadda and Rao, 1996; Waykar, 1998) Zhou *et. al.*, (1993) studied the histological changes in the ovotestes of snail *Biomphalaria glabrata* on exposure to molluscicides. Jyothi and Narayan (1996) studied the effect of organophosphorous insecticide phorate on gonads of freshwater fish *Clarius batrachus*. Kumari and Kumar (1997) studied the histopathological alterations in ovary induced by aquatic pollutants in *Channa punctatus*. Hazarika and Das (1998) investigated the histopathological changes induced in ovary of *Heteropneustes fossilis* exposed to BHC. Khan and Jha (2000) studied gonad histopathology of the freshwater fish, *Channa punctatus* after exposure to phosalone. Ramchandra Mohan (2000) studied malathion induced changes in the ovary of freshwater fish, *Glossobius quaris*. Baruah and Das (2002) studied histopathological changes in ovary of fish *Heteropneustes fossilis* exposed to paper mill effluent. Phirke (2008) studied the effect of quinolphos and thiodan on gonads of freshwater bivalve, *Parreysia corugata*. Otitoloju *et. al.*, (2009) studied the histopathlogy and bioaccumulation of heavy metals in ovotestes of giant land snail, *Archachatina marginata*. Kandasamy and Muthukumaravel (2010) studied the toxic effect of chromium on the histoarchitectural alterations in ovary of freshwater fish, *Oreochromis mossambicus*.

In the light of above researches, the present investigation was undertaken with a view to study the histopathological changes in the female gonad of *Lamellidens marginalis*, under cadmium toxicity.

MATERIALS AND METHODS

After collection of the animals from habitat, they were immediately transported to the laboratory. The fouling and mud on shell valves were removed without disturbing the siphonal regions. The equal sized animals (90-100 mm shell length) were grouped and kept in sufficient quantity of water (animal/liter) in aquaria with aeration for 24 hrs to adjust the animals in laboratory conditions (with renewal of water at interval of 12 to 13 hrs). No food was given during acclimation time and during experiments. After 24 hrs, 05 groups of animals of almost equal size (90-100 mm shell length) were formed and each group with 10 animals including control group and exposed to different test concentrations of cadmium for static bioassay tests. The stock solution of cadmium was prepared by was made dissolving appropriate quantity of cadmium chloride (CdCl2 2½H2O AR Grade CDH Bombay) in double distilled water. The pH of the water is brought between 6.9 and 7.1 by adding 1N HCl (due to insolubility of cadmium in reservoir water having 7.6 to 8.1).



Photograph showing: I from North to South and II. From West to East

Appropriate test concentrations were then prepared and animals were exposed. The experiments were conducted in natural day-night rhythm. The experiments were repeated three times for confirming observed LC_0 and LC_{50} values. The 96 hrs acute test was recorded. After 96 hrs acute toxicity test using cadmium for histological studies, gonad from control, LC_0 and LC_{50} groups were fixed in aqueous Bouin's Hollande fluid for 48 hrs. The dissected tissues were dehydrated with alcohol and toluene and embedded in paraffin wax (58 to 60°C). Gonad sections were cut down at 6-7µ. For histopathological study of gonad Mallory's triple stain was used. All the photomicrography was made under light-microscope Labo VT-20 Scan model.

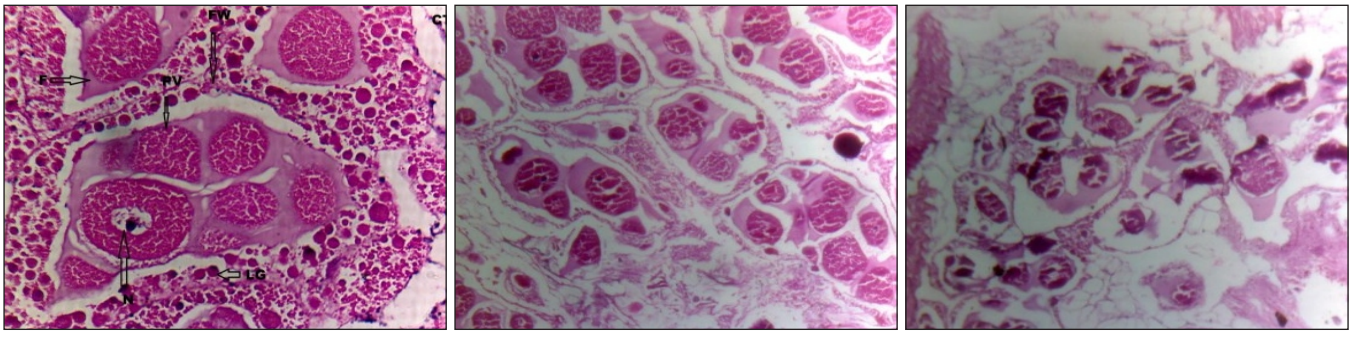
RESULTS AND DISCUSSION

The polluting substance could affect the histology of the organism, meaning the deterioration of tissue and damage at the cellular level. Severe damage at the cellular level was observed in female gonad after acute exposure. As compared to tissues of a control group of bivalve mollusks, these histological changes were prominent after acute exposure as compared to control group.

During the experimental period, the female gonad showed a developmental condition of gonad. In the control group, female follicle showed follicle wall with prominent germ cells and vitellogenic oocytes of different sizes. Many free

mature eggs possessed dense cytoplasm and distinct nucleus located in centre having prominent nucleolus. The staining of these different parts in female follicle was distinct (Fig.1). After acute exposure to cadmium LC_0 group and LC_{50} group of bivalves showed considerable damage to the female gonad as compared to control group. In LC_0 group, the follicle wall ruptured at places with shrinkage of germ cells along the wall. Deterioration of ooplasmic material, the nucleus and nucleoli were observed in mature eggs. The germ cells and vitellogenic oocytes lost their shape and detached from follicle wall. Swelling of follicle was considerable (Fig. 2).

In LC_{50} group, the follicle wall was distorted, mostly showed prominent nuclei, nucleoli and the cytoplasm was opaque. These are likely to undergo degeneration. Small sized previtellogenic oocytes also showed such conditions. Nature of damage was more severe in LC_{50} than the LC_0 group (Fig. 3).



1. Control group **2. LC_0 group** **3. LC_{50} group**

T. S. of Female gonad of *Lamellidens marginalis*

Fig. 1: Control group. **Fig. 2:LC_0 group.** **Fig. 3: LC_{50} group**
F: Follicle **MG:** Mature Gamete, **GO:** Growing Oocyte, **GC:** Germ Cell, **C:** Connective Tissue, **N:** Nucleus, **DF:** Distorted Follicle, **LST:** Loss of Stroma, **ST:** Stroma

Histological changes in gonads: (Fig 1to3):

Control
In female ovaries many vitellogenic oocytes were found and being released. In few follicles degenerating oocytes were also seen.

LC_0 and LC_{50} groups
In summer, LC_0 group showed the formation of many pre vitellogenic and vitellogenic oocyte. Prominent vacuole appeared in their nuclei and lipid globules and nutritive cells decreased much in quantity. In LC_{50} group, follicles distorted and ooplasmic material and nuclei showed vacuoles, vitellogenic oocyte were also formed. Prominent vacuole appeared in their nuclei and lipid globules and nutritive cells decreased much in quantity. In LC_{50} group, follicles distorted and ooplasmic material and nuclei showed vacuoles.

LC_{50} group showed swelling of the follicle walls and deterioration of cellular material. F ollicle wall was ruptured in places. Cytoplasm of oocytes showed prominent vacuoles. A few vitellogenic oocytes showed karyolysis and appeared to undergo degeneration. The vitellogenic oocytes showed fragmentation of cytoplasm. The damage was more severe than LC_0 group (Fig. 3). Any particular alteration of cell may indicate the presence of disease or the toxic substance. There is a clear correlation between pathological condition of cell or tissues and its affected functions (Brown *et al*, 1967). The extent of damage induced by the toxicant to a particular organ can also be judged on a cellular level. Pathological and biochemical disturbances in aquatic organisms like mollusc due to pesticide toxicity are well documented, Waykar and Lomte (2002 and 2004). Histopathological changes are mostly confined to organs directly involved in their metabolism and detoxification, Rashatwar and Ilyas (1994). In the control group, during maturation the lumen of a female follicle was filled with developing oogonia and primary oocytes. Few follicles showed primary germ cells along the wall. The nuclei of the oocytes and ripened gametes were conspicuous, but chronic stress from experimental group showed rupture of follicle at some places, discontinuous germ cells, loss of continuity in the follicle wall, dislocation of nucleus, internal follicular edema in ovary and disrupted basement membrane. Comparing the effects of Cadmium on female gonads of bivalves, it was observed that, the effects were more prominent in LC_{50} group, followed by LC_0 group. Follicular shrinkage and distortion was observed in LC_0, LC_{50} and chronic groups. In LC_{50}, vacuoles in cytoplasm and degeneration of oocytes were observed. Similar changes were observed in both LC_0 and LC_{50} groups of *V. bengalensis* after acute exposure to folithion and lebaycid (Muley, 1985) and in the female gonad of *Meretrix meretrix* after acute exposure to Cypermethrin (Prabhupatkar, 2004).

Most of the study is related to pesticide induced changes in the cytoarchitecture of different organs were carried out on fishes and other invertebrates, while that of mollusk and bivalves were still scanty (Shinde *et.al*, 2019). It is widely recognized that, growth and reproduction in aquatic animals were affected due to contamination of toxicants in aquatic

media. The pesticide shows the hazardous effect on the inhabitants of the aquatic species. There were histopathological changes in *Lamellidens corrianus* and *Lamellidens marginalis* exposed to Cythion Malathion from Godavari River, Paithan. The severity of effect of pesticide of both the species was more at LC_{50} than LC_0 from gonads (Muley and Mane, 1987).While studying alterations in gonad due to Endosulfan to freshwater bivalve, *Lamellidens marginalis* and *Lamellidens corrianus* from Godavari River, Paithan, similar trend of histopathological changes was observed and it was stated that, the pesticide stress affect the reproductive status (Muley and Mane, 1998). While studying the effect of carbaryl on fish, *Clarrius batrachus* gonad vacuolization and necrosis was observed in tissue (Jyothi and Narayan, 1999). Author observed similar changes in the present investigations also.

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