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Cypermethrin induced histopathological alterations in female Gonad of Estuarine Clam, *Katelysia opima* (Gmelin)

Madhura Mukadam* and Arvind Kulkarni

Department Of Zoology, Gogate Jogalekar College, Ratnagiri, India
Corresponding author

KEYWORDS

Cypermethrin, female gonad, *Katelysia opima*, histopathology

A B S T R A C T

This study aimed to investigate the effect of cypermethrin on the micro-architecture of the gonad in female estuarine clam, *Katelysia opima* (Gmelin). This was a randomized controlled experiment. The experiment was carried out on the clams collected from Bhatye estuary of the Ratnagiri district of Maharashtra state between February and May 2012. A total of 10 female estruarine clams of medium size 4.0 – 4.4 cm were selected. They were exposed to lethal (1.58 ppm) and sub-lethal (0.158 ppm) concentrations of cypermethrin for 4 (acute) and 30 (chronic) days, respectively. Sections of 5 to 6 µm thickness were stained with Ehrlich's Haematoxylin and alcoholic Eosin stain. Micrographs of the stained sections were taken using a Trinocular research microscope. The histolopathological findings include partial disruption of ovarian follicle vacuolation of germinal cell's cytoplasm and damaged interfollicular 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. Chronic exposure to Cypermethrin showed remarkabke changes in the female gonad which were dose dependent. Cypermethrin has the potential to induce toxic effects on micro-architecture of female gonads

Introduction

Estuaries are among the most productive environments in the world, by serving as feeding grounds, as nurseries for juveniles of economically important fish and invertebrate larvae, and by providing shelter for many types of benthic organisms. However, they also rank amongst the most contaminated areas. Pesticides are more commonly found in estuarine areas. They are mainly introduced into rivers via run-off and then may enter marine areas, particularly estuarine and coastal zones. These pollutants may have major ecological consequences and could endanger organism growth, reproduction and survival [1].

Pesticides are the major source of water pollution, as it eradicate the economically

important species either indirectly through breaking the biological chains or directly produces toxic stress and chemical changes. The pesticides find their way into lakes, estuaries and oceans and get concentrated in the water column and finally settle in the soil sediments, which alter the physical, chemical and biological processes of water bodies. Estuarine sediments serve as a cradle bed for valuable fauna and the real effect of pollution begins with the biology of these food species, their distribution, feeding, reproduction, biochemical pathways and their supporting food Predominantly, as a result of extensive application of pesticide, large scale mortalities of estuarine animals occurred widely. Clams are very sensitive to a wide variety of toxicants in water; various species of clams show uptake and accumulation of many contaminants or toxicants such as pesticides [2].

Due to accumulation of these pesticides in tissues produces many physiological, histological and biochemical changes in the estuarine fauna by influencing the activities of several enzymes and metabolites. The pervious histopathological studies in which fish exposed to pollutants revealed that fish organs are efficient indicators of water quality [3,4]. Therefore, it is necessary to study in detail the histopathological alterations in different organs of estuarine animals and thoroughly investigate them in order to assess the extent of damage.

Among the pesticides, pyrethroids are commonly used due to their high effectiveness, low toxicity to birds and mammals, and easy biodegradability. [5]. Cypermethrin is a highly potent and broad spectrum synthetic pyrethroid which is used extensively for pest control. Although it is not persistent in the environment, the excess use of this pesticide may result in its entry into natural waters through agricultural run-off and ultimately cause damage to non-target organisms such as fish [6-8]. Cypermethrin is a synthetic pyrethroid insecticide widely used against vegetable and fruit sucking aphids, mites and saw flies. Exposure to chemical pollutants may cause many molecular, biochemical changes in the fish which precede cellular and systemic dysfunctions. So that, if appropriate parameters are monitored, early warning signs of distress may be detected. Similarly pesticides are also known to cause various histopathological effects on the ovary of fish [9-13]. However, there has been little information on the histopathological impact of Cypermethrin on female gonad of *Katelysia opima.* It is one of the edible species and staple food of coastal people. But due to the property of bioaccumulation this species is susceptible for pesticide toxicity. Gills, Hepatopancreas and gonads are the primary target organs for toxic action. Therefore, the present investigation was undertaken with a view to studying the histopathological changes in the female gonad of *K. opima*, under Cypermethrin toxicity.

Materials and Methods

The Bhatye estuary is located in between 17° 20’ N and 73° 20’ W in the Ratnagiri district in the Konkan region and this is the major estuary on the west coast of Maharashtra state. The experimental clams, *K. opima* used for the present study were collected during the summer season *i. e.* period of February to May, 2012 from Bhatye estuarine region, Ratnagiri coast, of Maharashtra. The clams of medium size (4.0 – 4.4 cms) were selected, brought to the laboratory and stocked in the plastic containers containing filtered, aerated estuarine water, for 48 hours. Clams well acclimatized to the laboratory condition were grouped in tens and kept in plastic

containers containing 5 liters filtered estuarine water. For every experiment, a control group of clam was also run simultaneously. The toxicity tests were repeated three times and LC_0 and LC_{50} values were determined.

The regression equation between the log of concentration (X) and probit mortality (Y) were determined statistically for acute toxicity using the formula $Y= \alpha + \beta \log (x)$ and 95 % fiducial limits were established according to Finney (1971).Static bioassay tests were conducted for 96 hours by using Cypermethrin (25% EC). The LC_{50} values are determined by following the guidelines given by committee of toxicity tests with aquatic organism [14] and Probit Analysis Method [15].

The acclimated clams were exposed to lethal concentration (1.58 ppm) for 96h and sub-lethal concentrations (0.158 ppm) for 30 days. Simultaneously a control group of healthy clams was maintained under identical conditions. After studying 96 hours (acute) and 30 days (chronic) toxicity of Cypermethrin to *Katelysia opima,* female gonad of control, LC_0 and LC_{50} groups from acute exposure and chronic exposure were removed and fixed in a neutral buffer formalin for 48 hours for proper fixation.

Tissues were washed in distilled water, then dehydrated in Ethyl alcohol, cleared in Xylol and embedded in tissue mat (at 58-60 °C melting point) and then they were sectioned at 5 to 6 μm thickness on a rotary microtome (Erma, Japan). Theses Sections were stained with Ehrlich's hematoxyline and alcoholic Eosin stain and mounted in Distrene Plasticiser Xylene *(*DPX). All the observations for microphotography were done under Trinocular research microscope attached to the camera (Carl Zeiss, model: Axiostar).

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 and chronic exposure. As compared to tissues of a control group of clams, these histological changes were prominent after chronic exposure as compared to acute 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 Cypermethrin, LC_0 group and LC_{50} group of clams 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_0 than the LC_{50} group (Fig.3).

T. S. of Female gonad of *Katelysia opima*

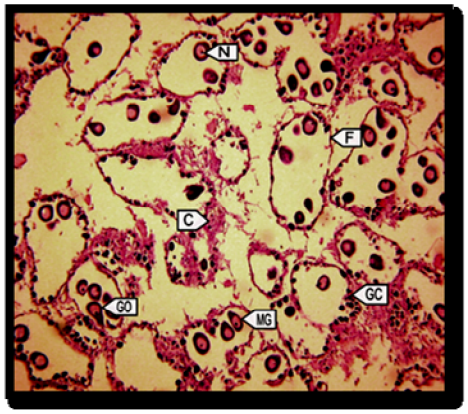

Fig. 1 Control group(HE 100X)

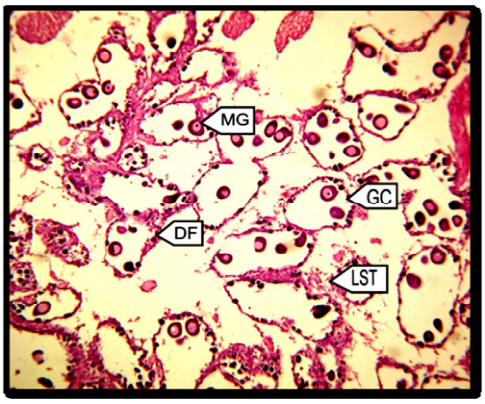

Fig. 2 LC_0 group(HE 100X)

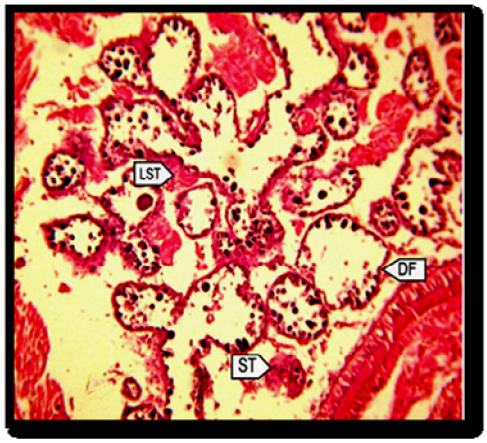

Fig. 3 LC_{50} group (HE 100X)

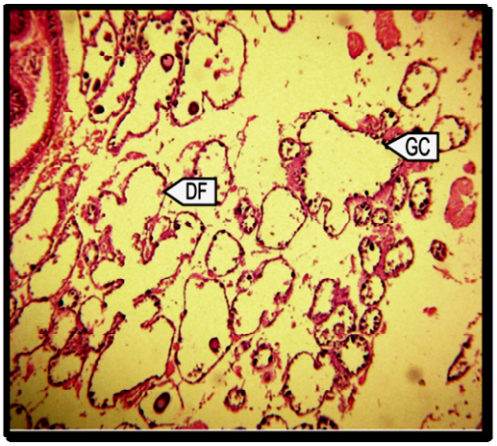

Fig. 4 Chronic group (HE 100X)

F : Follicle
GO : Growing Oocyte
C : Connective Tissue
DF : Distorted Follicle
ST : Stroma

MG : Mature Gamete
GC : Germ Cell
N : Nucleus
LST : Loss of stroma

Chronic group showed swelling of the follicle walls and deterioration of cellular material. Follicle 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 acute exposure (Fig. 4).

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 [16]. 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 [17,18]. Histopathological changes are mostly confined to organs directly involved in their metabolism and detoxification [19].

In the control group, during maturation of a female follicle in the lumen 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 Cypermethrin on female gonads of clams, it was observed that, the effects were more prominent in chronic group, followed by LC_{50} and LC_0 group. Follicular shrinkage and distortion was observed in LC_0, LC_{50} and chronic groups. In LC_{50} and chronic group, vacuoles in cytoplasm and degeneration of oocytes were observed. Similar changes in both LC_0 and LC_{50} groups of *V. bengalensis* after acute exposure to folithion and lebaycid [20] and in the female gonad of *Meretrix meretrix* after acute exposure to Cypermethrin [21].

Most of the study of related to pesticide induced changes in the cytoarchitecture of different organs was carried out on fishes and other invertebrates, while that of mollusk and bivalves were still scanty. 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 [22]. 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 observedstated that, the pesticide stress affect the reproductive status [23]. While studying the effect of carbaryl on fish, *Clarrius batrachus* gonad vacuolization and necrosis was observed in tissue [24]. Similar changes were observed in the present investigations

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