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RESEARCH ARTICLE

EFFECT OF THE NITRATE FERTILIZER UREA ON THE ULTRASTRUCTURAL CHANGES IN THE GILL OF FRESHWATER FISH CATLA

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ABSTRACT

Ultrastructural study of the gill of *Catla catla* on exposure to 10% LC_{50} sublethal concentration of nitrate fertilizer urea was carried out. Hypertrophy and hyperplasia of the lamellar cells in combination with epithelial lifting and edema were noticed. Necrosis of pillar system with lamella resulted in blood congestion and even an aneurism. Hyper secretion of mucous on the epithelium is to protect against environmental alteration was also determined. Infiltrated macrophages and leucocytes in the lamellar tissue were observed which was a compensatory repair response to tissue damages. Apoptotic condition of the cell is evident by clumping of chromatin, swelling of nucleus and mitochondria.

INTRODUCTION

Increasing agricultural production through fertilizer application has resulted in increasing number of fresh water system being impacted by the contaminants present in waste water releases (Figueiredo Fernandes *et al.,* 2006). The effects of pollutants on aquatic animals can be assessed by population studies, in particular through the evaluation of the survival rates and reproductive success. However, as in aquatic animals gills are directly exposed to the environment, they may also be used as indicators of water quality (Rankin and Jensen, 1998). Gills are multifunctional organs with a complex internal organization that is similar in most teleosts (Hughes, 1984 and Laurent, 1984).

Gills represent the largest body surface area of freshwater fish, and in the branchial epithelium, the distance between the water and blood is only a few micrometers (Hughes, 1984). This organ is both morphologically and physiologically complex, performing multiple functions, such as gaseous exchange, ion and water exchange, acid base balance, nitrogenous waste excretion, toxicant uptake, detoxification, excretion, and several other metabolic transformations (Wood and Soivio, 1991, Evans *et al.,* 2005 and Tang and Lee, 2011). Due to its multiple regulatory functions, delicate structure, and constant exposure to the external environment, gills are the most sensitive target organ of waterborne pollutants (Lauren and McDonald 1987a b, Perry and Laurent 1993 and Pelgrom *et al*., 1997). Indeed, histopathological changes are the result of the integration of a large number of interactive physiological processes (Van de Oost *et al.,* 2003). Histopathological changes have been widely used as biomarkers in the evaluation of the health of fish exposed to contaminants, both in the laboratory and field studies (Teh *et al*.,1997, Thophon *et al*.,2003 and Kasherwani *et al*.,2009).The ultrastructure of tissues and organs is altered when the waterborne contaminant is still at low levels. Therefore, histopathological assays may provide a valuable screening method before severe damage occurs (Jiraungkoorskul *et al.,* 2007). Hence in the present investigation transmission electron microscopic study was carried out to assess the architectural changes in the cells of the gill of *Catla catla* on exposure to the nitrate fertilizer urea.

MATERIALS AND METHODS

The fish, *Catla catla* fingerlings (Weight :10g ; Length 8 cm) were collected from the Katherasan Aqua Farm near Thanjavur, Tamil Nadu. They were acclimatized for 15 days in large cement tanks (Temperature – 28 ± 2°C**;** total hardness – 518 ± 23 mg/l; DO - 5.6 ± 0.2 mg/l; salinity - 1.2 ± 0.13 ppt and pH - 7.8 ± 0.04) previously washed with 1% potassium permanganate. The water as renewed every 24 h. The LC_{50} of urea for 96h was found out by using Probit method (Finney, 1971).

For transmission electron microscopic studies, the gill tissue of control and 10% chronically urea treated fish *Catla catla* were collected in 2.5% buffered glutaraldehyde. The tissues were cut into 3 x 3 mm pieces and kept in glutaraldehyde at 4°C overnight. They were then post fixed in 1% buffered Osmium tetraoxide for 2 hours at 4°C. The tissues were washed in the same buffer before and after fixation with osmium tetraoxide. Then the tissues were treated with graded series of alcohol viz.

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30%, 50%, 70%, 80%, 90% and 100% for 10 minutes each. This was followed by treatment with propylene oxide twice for 10 minutes each. The tissues were then infiltrated with Taab 812 epoxide embedding resin at 20%, 50%, 75% and 100% concentrations with propylene oxide for 2 hours each. Finally the tissues were embedded in the same resin mixture with added catalyst and cured at 60°C for 48 hours. The blocks obtained were trimmed and semi thin sections were cut with glass knives using LKB ultra microtome. Then the sections were stained with toludine blue and screened under the light microscope to look for areas of interest. Ultrathin sections were cut using microstar diamond knife. The sections were stained with uranyl acetate and lead citrate. Stained sections were viewed in JEOL, JEM 100 SX Transmission electron microscope at an accelerating voltage of 60 or 80 KV.

RESULTS

The LC_{50} value based on probit analysis was found to be 125.67 mg/l for 96 h of exposure to urea. In the present investigation, the transmission electron micrograph of the control gill lamella possessed a central vascular axis consisting of the pillar system. The pillar system is a collective term for the pillar cells and blood capillaries (Fig.1). The pillar cells are the main body of this system which supports the lamella. The pillar cell has a target nucleus occupies the greater part of the main cell body with irregular outline and heterochromatic clumps. The cytoplasm has few mitochondria and poor endoplasmic reticulum. Four cytoplasmic projections known as pillar arms, two arms on each side of the cell extend to overlap with the arms of the next pillar cell to form a space; (blood lacuna) in which blood cells move(Fig.2&4). The pillar system is coated with a basal lamina (BL) and loosely arranged interstitial tissue that contained pericytes (PCTs). The protruding region of the lamellae is composed of pavement cells (PVCs). The superficial layer of the gill filament epithelium consisting of mucous cell(MCs), mitochondria rich Chloride cells, their precursors and intercalating support cells (SCs) which are also externally covered by a monolayer of pavement cells (PVCs). The deep layer of the lamella was latero-basally lined by myoepithelial cells (MECs) and basal lamina, formed by a network of undifferentiated cells (UDCs), enclosed with neuroepithelial cells (NECs), and eosinophil-like cells.

Significant ultrastructural alterations appeared in the gills of fish exposed to urea. The primary and secondary gill lamellae exhibited hypertrophy and hyperplasia of the epithelial cells partial fusion of some secondary lamellae, lamellar aneurism, besides epithelial lifting and edema (Fig.2). The pavement cell appeared irregular with a considerable loss of micro ridges (Fig. 2). Vasodilatation in many areas of the secondary lamellae with breakdown of the pillar cell system appeared by degenerative and necrotic changes of the pillar cells (Fig.3). Furthermore congestion of blood spaces by erythrocytes with presence of different leucocytes has been observed (Fig.2). Occasionally, proliferation of chloride cells and mucous cells could be identified in the secondary lamella (Fig.2). The mucous cells were completely filled with mucous containing vacuoles (Fig.4). The swelling of the inter-cellular spaces between the epithelial lining and the basal lamina of the respiratory gill lamellae led to the appearance of wide spaces from the base towards the tip of the gill lamellae Infiltration of large number of eosinophilic granular cells into the intercellular tissue was evident (Fig.2).

Hyperplasia of the gill filament epithelium was the dominant change recorded in the gills exposed to sublethal concentration of urea (Fig.2). Hyperplasia was often accompanied by extensive proliferation of both mucous and chloride cells. Clusters of chloride cells were evident, some of them showing signs of degeneration. In addition inflammatory cells including lymphocytes and macrophages were widely accumulated (Fig.2 &6)

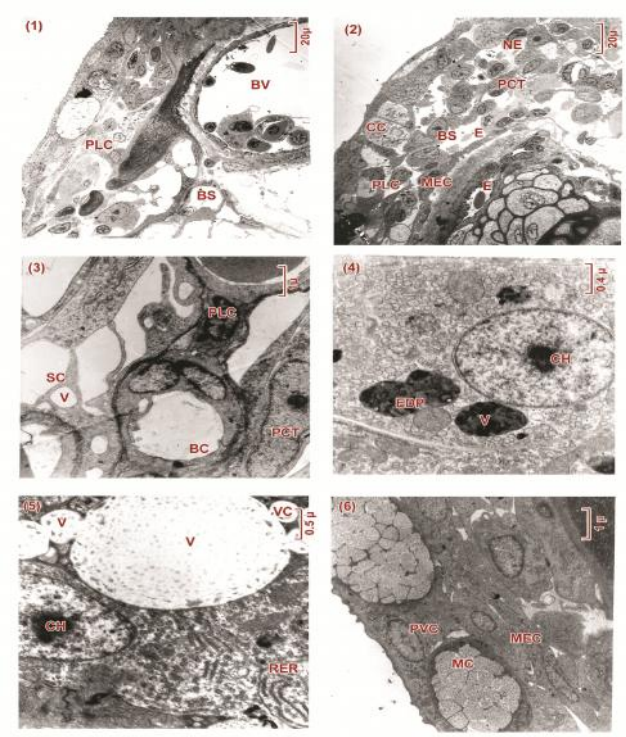

Fig. 1-6 Transmission electron micrographs of the gill of *Catla catla* exposed to sublethal concentration of urea at different durations

PLC	-	Pillar cell
PV	-	Blood vessel
BS	-	Blood space
V	-	Vacuole
MR	-	Microridge
PVC	-	Pavement Cell
MC	-	Mucous cell
SC	-	Intercalating support cell
UDC	-	Undifferentiated cell
E	-	Erythrocyte
LC	-	Leucocyte
MEC	-	Myoepithelia cell
PCT	-	Pericytes
CC	-	Chloride cell
EDP	-	Election dense particles
CH	-	Chromatin
N	-	Nucleus
SV	-	Smaller vesicles
RER	-	Rough endoplasmic reticulum

In the present study, clumping of chromatin, Swelling of nucleus, mitochondria, membrane bound fragments of cells, fusion of smaller vesicles into large vacuoles (Fig. 4 &5). These ultrastructural alterations are corresponds to the apoptotic cell.

DISCUSSION

Fish gills perform numerous functions such as respiration, osmoregulation, excretion of nitrogenous waste products and acid base balance. Because of their vulnerable external location and large surface area, they are often a major target of pollutants in water (Daoust *et al*., 1984).

In the present investigation, the electron micrograph of the control gill, the superficial layer of the filament epithelium (Fig. 1) contained mucous cells (MCs), mitochondrion-rich cells (MRCs), their precursors, and intercalating support cells (SCs), which were externally covered by a monolayer of pavement cells (PVCs). The deep layer (Fig. 1), which was latero-basally lined by myoepithelial cells (MECs) and basal lamina, was formed by a network of undifferentiated cells (UDCs), and also enclosed neuroepithelial cells (NECs), eosinophil-like cells, and MC precursors. Each lamella possessed a central vascular axis, the endothelium of which was composed of pillar cell (PLC) cytoplasmic extensions, externally coated with a basal lamina and a loose interstitial tissue that contained pericytes (PCTs). In the protruding part of the lamellae, the most external coat was composed of lamellar PVCs (Fig. 1).

The predominant ultrastructural changes noticed in the urea exposed fish are hypertrophy and hyperplasia of the epithelial cells, partial fusion of some secondary lamellae, lamellar aneurism, besides epithelial lifting and edema (Fig.2). This may be early responses of the gills to the harmful substances. These alterations are examples of defense mechanisms because the lifting lamellar epithelium and edema increased the distance between the external environment and the blood, thus serving as a barrier to the entrance of contaminants (Fernandes and Mazon, 2003 and Sorour and Harbey, 2012).

Gill hyperplasia has been regarded as a common sign of chronic toxicity caused by various chemical pollutants. In the present study, gill hyperplasia was noted in the fish exposed to urea toxicity (Fig.2). It was suggested that such a hyperplastic reaction may increase the epithelial thickness so as to retard or prevent the entry of toxic, ions into the blood stream or to compensate for osmotic imbalance (Laurent, 1984). In the gill lamella of urea exposed fish of the present study, hypertrophy of chloride cells and hyperplasia of the mucus cells were observed (Fig.2,6). These observations fall in line with that of Powell *et al*. (1995) and Monteiro *et al.* (2012) they have suggested that hypertrophy of the chloride cells correlated with the increased secretory activity. Hyperplasia of mucus cells is to hyper secretion of mucous. The polyanionic mucin trap toxic environmental substances, mucous hyper secretion would prevent pollutant from crossing the epithelium. Kumar and Pant, (1981) suggested that the increased mucous secretion under toxic conditions is known to bar the entry of toxicants into the fish, probably by interacting with toxic ions and then resulting in the formation of a film of coagulated mucus on the surface of the gill, or by providing a physical barrier for macromolecules. In the present investigation the intercellular spaces contained leukocyte-like cells and macrophages with large residual digestive vacuoles (Fig. 2&4). The major ultrastructural alterations in the gills of fish exposed to the nitrate fertilizer urea showed a distinct degeneration and necrosis of pillar cells and also have damaged the capillary walls of the secondary gill lamellae (Fig.3). Similar observations had been recorded by Schwaiger *et al*. (2004) they have implicated that these gills alterations might interfere with normal respiratory functions and might lead to an impairment of the general health status of fish. Damaged pillar cells can result in an increased blood flow inside the lamellae, causing dilatation of the marginal channel, blood congestion or even an aneurism (Rostey-Rodriguez *et al*., 2002 and Camargo and Martinez, 2007). In the present study there is a reduction in the number of micro ridges of the pavement cells in the gills of urea treated fish (Fig-2). Wong &Wong (2000), Mazon *et al*. (2002) and Biagini *et al*. (2009) have also observed reduction of ridges. Mallat (1985) had said that the micro ridges are related with the retention of mucous on the epithelium as a way to protect it against environmental alterations. Increase in mucous containing vacuoles in the mucous cells are apparent to the mucous function in protecting the gill epithelium from environmental impacts, infectious agents, toxic agents and particles in suspension (Powell *et al*., 1992 and Biagini *et al*., 2009). Perry and Laurent (1993) stated that mucous cells can be efficient in seizing the toxic agents and thus help in preventing the entry of toxic substances into the gills. In the present study the infiltration of macrophages and leukocytes in lamellar tissue was noticed (Fig.2). The swelling of the intercellular spaces and infiltration of leucocytes was parallel to those alterations seen by Hoda and Hanan (2003). It was suggested that the infiltration of these cells is a compensatory repair response to tissue damage that occurs on exposure to different types of pollutants (Wendelaar Bonga and Lock 1992, Dutta *et al*., 1996, Teh *et al*., 1997 and Li *et al*., 1998). In the present investigation the cellular fragments were comparable morphologically to those observed in apoptosis. Clumping of chromatin, Swelling of nucleus, mitochondria (Fig.4&5). These observations were favoured by the following authors Kerr *et al.*, 1972, Daoust *et al*., 1984 and Monterio *et al.*, 2012. Apoptosis is well suited to a role in tissue homeostasis: it can result in extensive deletion of cells with little tissue disruption; there is no inflammation, as is elicited by coagulation necrosis; and the process permits the reutilization of cellular component.

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