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Studies on the Impact of Heavy Metal Copper on The Ultrastructure of Gill in the Mangrove Crab *Sesarma brocki*

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Abstract

Ultrastructural alternations in the in the gill architecture of the mangrove crab *Sesarma brocki* were observe on chronic exposure to 10% and 30% of the 96hr LC_{50}-value (10.91 mg/l) of copper. In the gill tissue of control crab gill epithelial cell and pillar cell nucleus were having dispersed chromatin, abundance of mitochondria was visualized. In the gill cells of low toxicity exposed crab condensation of chromatin, swelling of mitochondria and numerous vesicles were seen. High sublethal toxicity inflicted crab gill tissue revealed fragmentation of chromatin and infiltration of nephrocytes also called as podocytes into the haemolymph spaces were observed. The present remarkable ultrastructural alterations on exposure to copper could serve as "Biomarker" for assessing heavy metal toxicity in the aquatic environment.

Keywords

Sesarna brocki, Copper, Gill, TEM.

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INTRODUCTION

Crustacean gills are the first organ exposed to pollutants when ambient water is polluted (Wu & Chen, 2004). They are known to perform multiple physiological functions. In addition to being the organ of respiratory gas exchange (Dejours, 1981), they are also responsible for hemolymph acid-base regulation (Truchot, 1978;Truchot, 1979) and nitrogen excretion (Kormanik & Cameron, 1981). On cellular bases, the epithelial cells of crustacean gills play a chief role in the ion-regulatory processes (Pequeux, 1995) and (Masui et al., 2005). While thin epithelium predominates in anterior gills, posterior gills possess thick cells or ionocytes whose membrane has the dense apical enfolding and basolateral invaginations involved in ionic transport (Copeland & Fitzjarrell, 1968; Henry & Wheatly, 1992; Pequeux, 1995) and (Towle & Kays, 1986). Laporte et al. (2002) studied the histopathological changes of gill in the shore crab Carcinus maenas exposure to the methylated mercury. Abdel Mohsen (2016) observed condensation of chromatin in the nucleus and abnormal shape of mitochondria in the gill lamellar cells of Penaeus japonicus. Yamuna et al. 2009 observed elevated cuticle and thickening of epithelium in gill of prawn Macrobrachium malcolmsonii. The present investigation has been in an attempt to elucidate the alterations in the ultra-structural architecture of the gill of Sesarma brockii on heavy metal infliction.

MATERIALS AND METHODS

The mangrove crab Sesarna brocki (Carpace length 3-4cm) were obtained from fisherman near Muthupet mangrove. They were acclamatized to lab condition with estuarine water. The LC_{50} value of Copper for 96h was determined by using Probit method (Finney, 1971). For transmission electron microscopic studies, the gill tissue of control and treated crab *Sesarma brockii* 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. 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 micro star 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 AND DISCUSSION

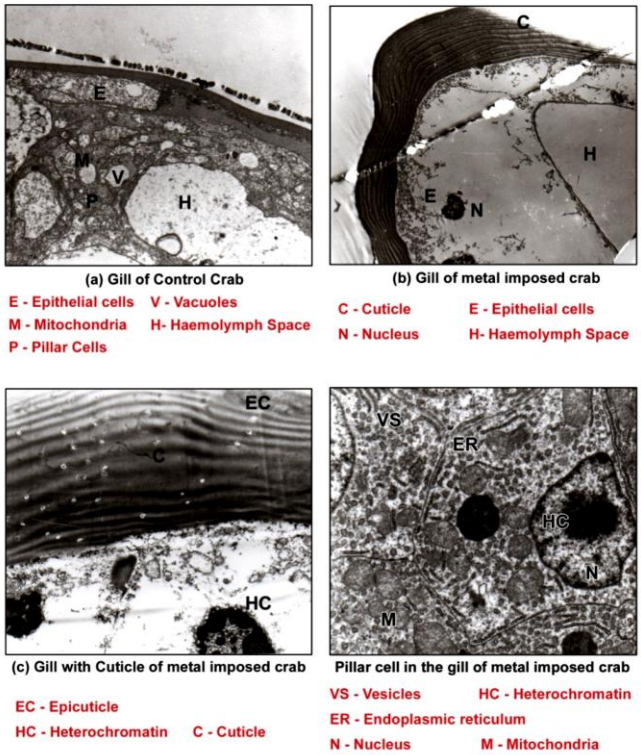
Crabs are economically important because they are used as an alternative source of food for the people of coastal regions. Heavy metal and their salts containing effluents are directly discharged into the aquatic environment by many industries. Heavy metal pollutants are a major problem in aquatic environment because of their persistency and tendency to accumulate in organisms and undergo food chain amplification. In the present study toxicity evaluation of heavy metal copper caused 50% mortality of *Sesarma brockii* at 96 hours was 10.91 ppm. The LC_{50} values of copper for 24, 48, 72 and 96 hours were 11.38, 11.21, 11.07 and 10.91 ppm respectively. The determination of LC_{50} values are highly useful in the evaluation of safe level of tolerance to pollutant and it provides fundamental data for the design of more complex disposal models. The present investigation reveals that the LC_{50} values decreased with increased period of exposure and rate of mortality increased as the concentration of heavy metal increases. These results indicate that the effect of heavy metal is dose dependent.

Transmission electron microscopic structure of the gill of heavy metal treated *Sesarma brockii* are presented in the plate 1 and 2. The TEM of control crab illustrate the cytological architecture of gill tissue with the cuticle and underlying chief cells. The cuticle comprises three layers; a thin epicuticle, exocuticle and a multilayered endocuticle. In the toxicity exposed gill, the cuticle is found to be elevated. The epithelial cells and pillar cell nuclei are having dispersed chromatin. Abundance of mitochondria are visualized in the control crab. In the less toxicity exposed crab gill tissue condensation of chromatin swelling of mitochondria and numerous vesicles are seen. High sub lethal inflicted crab revealed fragmentation of chromatin, infiltration of nephrocytes also called as podocytes into the haemolymph spaces are seen.

Gills are the first defense line of tissue in aquatic organism against the pollutants in its surrounding medium and hence they are expected to be intensely affected. The gills of crustaceans are located in a key physiological position in connection with contamination from the surrounding water. The larger area of this organ forms a biological barrier between polluted medium and the internal compartment. In the present investigation the cuticle layer was found to be elevated in the copper toxicity-imposed crab. It has been suggested that the elevation of cuticle could be the structural alteration to withstand the toxic effect. Couch (1977), Nimmo et al. (1977) and Yamuna et al. (2009) observed similar pathological response in the gill architecture. In the nucleus of gill tissue of lesser heavy metal concentration treated crab of the present study there was general condensation and marginalization of chromatin was noticed and it might have been a structural adaptation to protect the DNA. The above observation is in harmony with that of Harman (1991) and Abdel Mohsen (2016). In the present investigation in the gill tissue of high sub lethal toxicity imposed gill there is swelling in the mitochondria and their reduction, increased number of vesicles with electron dense matrix were visualized. Yamuna et al. (2009) suggested that the operation of mitochondrial pumps was pretentious, which in turn could have reduced the uptake of available ions to counter effect the toxicity. Increased density of vesicle may envisage the secretary activity of the cell possibly the stress proteins. In the present study infiltration of nephrocytes (podocytes) into the haemocoel spaces

was observed. The detoxifying role of nephrocytes in many invertebrates viz., molluscs, arthropods, echinoderms when imposed to heavy metals was evident from earlier reports (Meyhofer *et al*., 1985; Meyhofer & Morse, 1996; Welsch & Rehkamper, 1987).

PLATE-1



(a) Gill of Control Crab
E - Epithelial cells V - Vacuoles
M - Mitochondria H- Haemolymph Space
P - Pillar Cells

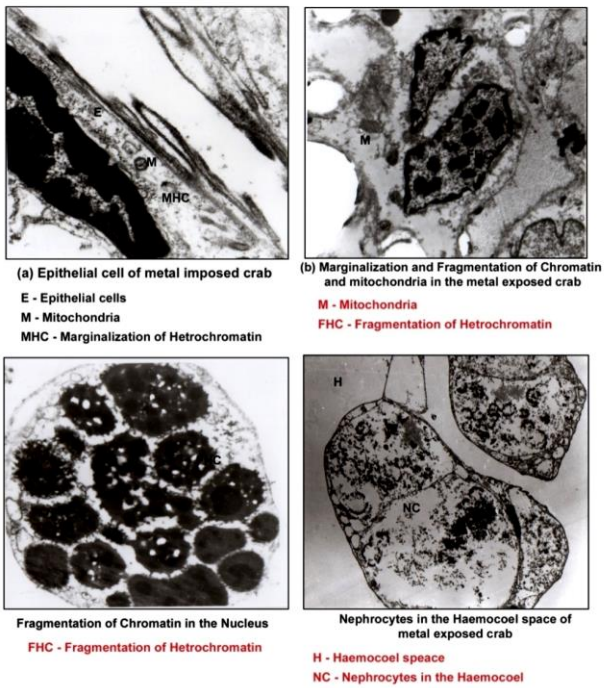
(b) Gill of metal imposed crab
C - Cuticle E - Epithelial cells
N - Nucleus H- Haemolymph Space

(c) Gill with Cuticle of metal imposed crab
EC - Epicuticle
HC - Heterochromatin C - Cuticle

Pillar cell in the gill of metal imposed crab
VS - Vesicles HC - Heterochromatin
ER - Endoplasmic reticulum
N - Nucleus M - Mitochondria

Plate 1: Transmission electron microscopic structure of the gill of heavy metal treated *Sesarma brockii*

PLATE-2



(a) Epithelial cell of metal imposed crab
E - Epithelial cells
M - Mitochondria
MHC - Marginalization of Hetrochromatin

(b) Marginalization and Fragmentation of Chromatin and mitochondria in the metal exposed crab
M - Mitochondria
FHC - Fragmentation of Hetrochromatin

Fragmentation of Chromatin in the Nucleus
FHC - Fragmentation of Hetrochromatin

Nephrocytes in the Haemocoel space of metal exposed crab
H - Haemocoel speace
NC - Nephrocytes in the Haemocoel

Plate 2. TEM of control *Sesarma brockii* illustrates the cytological details.

CONCLUSION

The present ultrastructural studies of the gill tissue of heavy metal-imposed mangrove crab Sesarma brocki revealed marked alterations in ultrastructural architechture. The severity of histological alterations correlates with the concentration of imposed copper. In the gill cells of low toxicity exposed crab condensation of chromatin, swelling of mitochondria and numerous vesicles were seen. Infiltration of nephrocytes were observed in the haemolymph spaces of higher toxic concentration exposed crabs. These ultrastructural alterations could serve as "Biomarker for assessing the aquatic toxicity.

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