World Journal of
Current Scientific Research

Journal Homepage: www.wjcsr.com

Toxic Effect of Cadmium Chloride on The Epidermal Club Cells and Mucous Cells of *Ompok bimaculatus*

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ARTICLE INFO

Keywords:
Cadmium Chloride
Club Cells
Epidermis
Mucous Cells
Ompok bimuculatus
Toxicity
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ABSTRACT

Effect of sub-lethal concentration of 29.25mg/L of the cadmium chloride on the club cells and mucous cells in the epidermis of *Ompok bimaculatus* has been studied. Effect of hazardous chemicals on the epidermis of fish may be reflected by change in histological organization and histochemical characterization of club cells and mucous cells *Ompok bimaculatus* is a fresh water butter catfish belonging to the family Siluridae and order cypriniforms. Cadmium is a naturally occurring toxic heavy metal with common exposure in industrial work places, plant soils and from smoking which causes health hazard to living organisms. The club cells and mucous cells in the epidermis of *Ompok bimaculatus* are main cellular constituents of epidermis, display proteinaceous reaction and stain weakly for glycoproteins. In the epidermis of *Ompok bimaculatus* these cells at different durations of sub-lethal concentration of Cadmium chloride show enlargement in size secreting their contents into intercellular spaces. At exposures where superficial layer epithelial cells are lost as exfoliation, the club cells with broken cell membrane also release their contents to the surface. It is possible that the club cell and mucous cells contents released into intercellular spaces may serve to plug intercellular channels, preventing the indiscriminate entry of the fluids in the environment surrounding the fish that might be initiated due to disruption of superficial layer of epidermis under influence of heavy metal cadmium chloride.

1. Introduction

Cadmium is a non-essential non-biodegradable element with no known biological function and is reported to be a major contaminant of aquatic ecosystem causing adverse effects on aquatic organisms (Hollis et al. 1999). It is released from diverse sources such as electroplating, paper, PVC plastic pigments and ceramic industries, battery, mining and smoldering units and other modern industries (Gupta et al. 2003). Signs of cadmium poisoning in fishes includes impaired gill function (Majewski and Gills, 1981), haematological changes (Lasson,1975; Johann et al, 1978) and distribution in osmotic ionic balance and carbohydrate metabolism (Lasson, 1975; Lasson et al ,1976 Lasson and haux,1982). The epidermis constitutes the boundary tissues of the fish and being continuously hydrated, unkeratinized and covered by a layer of slimy coating is more vulnerable to water borne toxicants. In this context the present study is an attempt to elucidate the toxicopathological effects of heavy metal salt cadmium chloride on club cells in the epidermis of a column feeder, carnivorous predatory fish *Ompok bimaculatus* belonging to the family Siluridae and order Cypriniformes.

2. Materials and Methods

Specimens of *Ompok bimaculatus* were collected from local ponds at Singramau, India (2019) and were acclimated to laboratory conditions for 15 days before starting of experiments. The fishes were fed with minced goat liver on alternate days. The feeding was stopped 24 hours before beginning of experiments and fishes were not fed throughout the experiments.

Ompok bimaculatus were exposed to sub-lethal concentration of 29.25mg/L of cadmium chloride. Fishes kept under control and experimental conditions were cold anesthetized following Mittal and Whitear's method(1978) and skin pieces from dorsolateral sides of the fish were excised, rinsed in saline water and were fixed in 10%neutral formalin and Bouin's aqueous at 3hour(h), 6h, 12h, 1day(d), 2d, 3d, 4d, 5d, 6d, 7d, 8d, 9d and 10d intervals.

Cite this article as Anurag Singh, 2021. Toxic Effect of Cadmium Chloride on The Epidermal Club Cells and Mucous Cells of *Ompok bimaculatus*. World J.Cur. Sci. Res.,1(3):185-190.

Standard methods of dehydration, clearing and embedding were used ,paraffin sections were cut at 5µm and were stained with Ehrlich's haematoxylin eosin to see general organization of the epidermis. The sections were subjected to histochemical techniques for glycoproteins and proteins following Lillie(1954),Bancroft and Stevens(1982) and Pearse (1985).

3. Results and Discussion

3.1 Control

The epidermis is divided into an outermost layer, a middle layer and a basal layer(PLATE Ia) .It is mainly composed of epithelial cells ,mucous cells and club cells.(PLATE Ia) . The superficial layer and middle layer of epithelial cells are polygonal while basal layer epithelial cells are cuboidal in outline. The superficial layer epithelial cells stain weakly for neutral glycoproteins as indicated by weak magenta colour reaction with AB/PAS (Alcian Blue/Periodic Acid, Schiff Stain). The free margins of these cells show stronger reaction for neutral glycoproteins .The epithelial cells in middle and basal layers show moderate positive reaction for acidic glycoproteins .These cells display strong reaction reactions for general proteins, basic proteins and protein bound $-NH_2$groups. Flask shaped mucous cells are found in superficial layer opening to the surface through the minute pores. The mucous cells underlying these flask shaped mucous cells are rounded occupying outer middle layer of the epidermis .The mucous cells stain strongly for acidic glycoprotein moieties indicated by strong greenish blue reaction with AB at pH 1.0 and at pH 2.0 and AB/PAS sequence. The mucous cells stain weakly for general proteins, basic proteins and protein bound –NH2 groups. In general club cells are arranged in two rows. These are voluminous ,appear rounded, oval or somewhat elongated and binucleated (PLATE Ia).These cells show weak reaction for acidic glycoproteins and strong reaction for general proteins, basic proteins and protein bound –NH2 groups and basic proteins.

3.2 Cadmium Chloride Treatment

Exposure to sub-lethal concentration of Cadmium chloride showed following histopathological alterations in various cellular components of the epidermis.

At 3 hr treatment vacuoles were observed in the epithelial cells throughout the epidermis and flattening of nuclei was observed. Eosoinophilic granular cells were observed (PLATE Ib). At 6 h exposure eosinophilic granular cells were frequently observed, epithelial cells undergo degeneration with prominent intercellular spaces.(PLATEIc). At from 12 h to 4 day treatments epithelial cells were extremely damaged. Intercellular spaces were quite large. Vacuoles were seen (PLATE Id). At 5 and 6 day treatment wear and tear in superficial layer was observed. In further treatment up to 10 day epidermis was observed in extremely damaged condition (PLATE Ie). There was appreciable increase in the intensity of reaction for neutral glycoprotein moieties in epithelial cells in different exposures. Increase in intensity for general protein, basic protein and protein bound –NH2 group was also observed in epithelial cells in various exposures.

At 6h and 12h decline in mucous cell density and increase in dimension was observed and they stain for neutral glycoproteins (PLATE If). At 1d, increase in mucous cell dimension was recorded (PLATE IIb). The main body of mucous cells stained for acidic glycoproteins while neck region stains for neutral glycoproteins. At 2d, there was decreased in mucous cell dimension and in crease in density. They displayed reaction for mixed glycoprotein moieties. At 3d mucous cells appeared rounded quite voluminous and few in number (PLATE IIc)). These stain for acidic glycoprotein moieties. At 8 day increased in mucous cell density and decreased in dimension and at 9 day and 10 day decreased in mucous cell density and increased in dimension was observed. These stained for acidic glycoprotein moieties.

At 3 h, the club cells showed enormous increase in their dimension with increase in eosinophilic content. At 6 h flattening in club cells was observed (PLATEIc). At 12 h and 1 day treatments the club cells appeared healthy with distinct membranous covering. At 2 day, 3 day and 4 day treatments the club cells got enormously increased (PLATEId). Their membranous covering showed disintegration releasing their contents into intercellular spaces between epithelial cells . From 5 day to 10 day treatments the disintegrating club cells open to the surface due to exfoliation of surface layer epithelial cells (PLATEIe). From 3 h to 4 day treatments, these cells showed appreciable reaction for neutral glycoproteins indicated by magenta colour reaction with AB/PAS (PLATEIIa,b,c). At 5 day and 6 day treatments these cells showed greenish blue reaction with AB/PAS showing reaction for acidic glycoproteins (PLATEIId). Again from 7 day to 10 day treatments these cells showed strong magenta colour reaction with AB/PAS suggesting presence of neutral glycoproteins (PLATE IIe).

At most of the exposures at sub-lethal concentration of cadmium chloride mucous cells look voluminous attaining greater size with heavy vacuolization in their glycoprotein moieties signifying increased mucous production an adaptation for protection assisting the fish to adjust to changed environment. Decreased mucous cell density at most of the exposures suggest that most of the mucous cells after release of their contents to the surface forming slimy coat get exhausted and are at rest resulting diminished mucous cell density.

At 3h,2d and 8d of sub-lethal exposures no significant decrease in the mucous cells suggest that mucous cells had revived their capacity at these exposures.

In the opercular epidermis of a catfish *Rita rita* exposed to dodecyl benzene sodium sulfonate, Roy (1988) reported that mucous cells showed a decrease in their diameter throughout the experiment and an increase in their diameter was observed only at 3 hour. Garg and Mittal (1993) have also observed increase in

Plate I

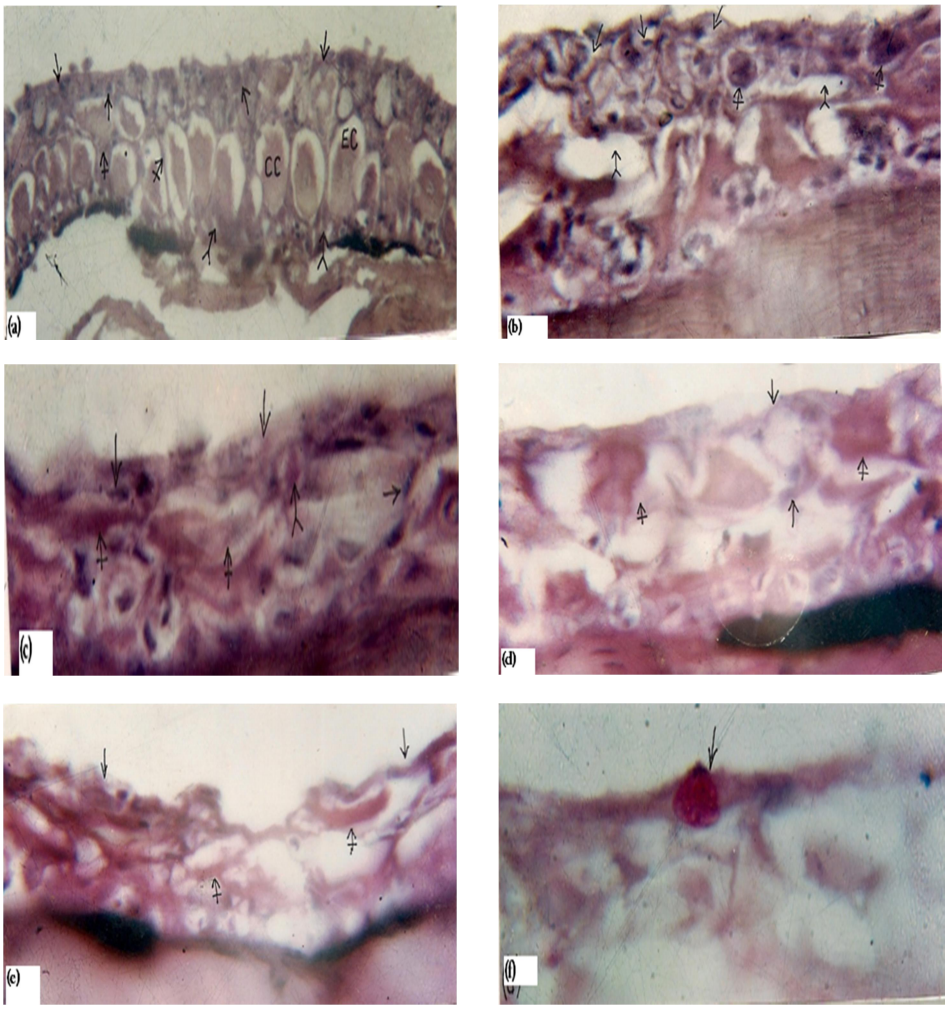

PLATE I (a –f)- Photomicrographs of the cross section of the epidermis of *Ompak bimaculatus* at control and at different duration of the cadmium chloride treatment.

(a) Showing polygonal epithelial cells in superficial and outer-middle layer (arrow), vertically elongated cells in deeper layer (barred arrow) and cuboidal epithelial cells in the basal layer (winged arrow). Note large sized uninuceleated club cells (CC), arranged in two rows with eosinophilic content (EC), showing shrinkage (normal, HE) x 600

(b) Showing flattening in nuclei of epithelial cells (arrow), appearance of acidophil cells (barred arrow). Also note intercellular spaces (winged arrow) (sub-lethal, 3h, HE)x1000.

(c) Showing degeneration of epithelial cells (arrow), flattening of club cells (barred arrow) and appearance of eosinophilic cells (winged arrow) (sub-lethal, 6h, HE)x1000.

(d) Showing heavily degenerated epithelial cells (arrow). Note enlarged club cells with broken cell membrane releasing their content to surface and intercellular spces (barred arrow) (sub-lethal, 3d, HE) x 1000.

(e) Showing disintegration and exfoliation of surface layer epithelial cells (arrow). Note damaged club cells (barred arrow) opening to surface (sub-lethal, 5d, HE)x1000.

(f) Showing abrupt decline in mucous cell density. Note enlarged mucous cell with neutral glycoprotein content (arrow) (Lethal, 3h, AB/PAS) x 600.

Plate II

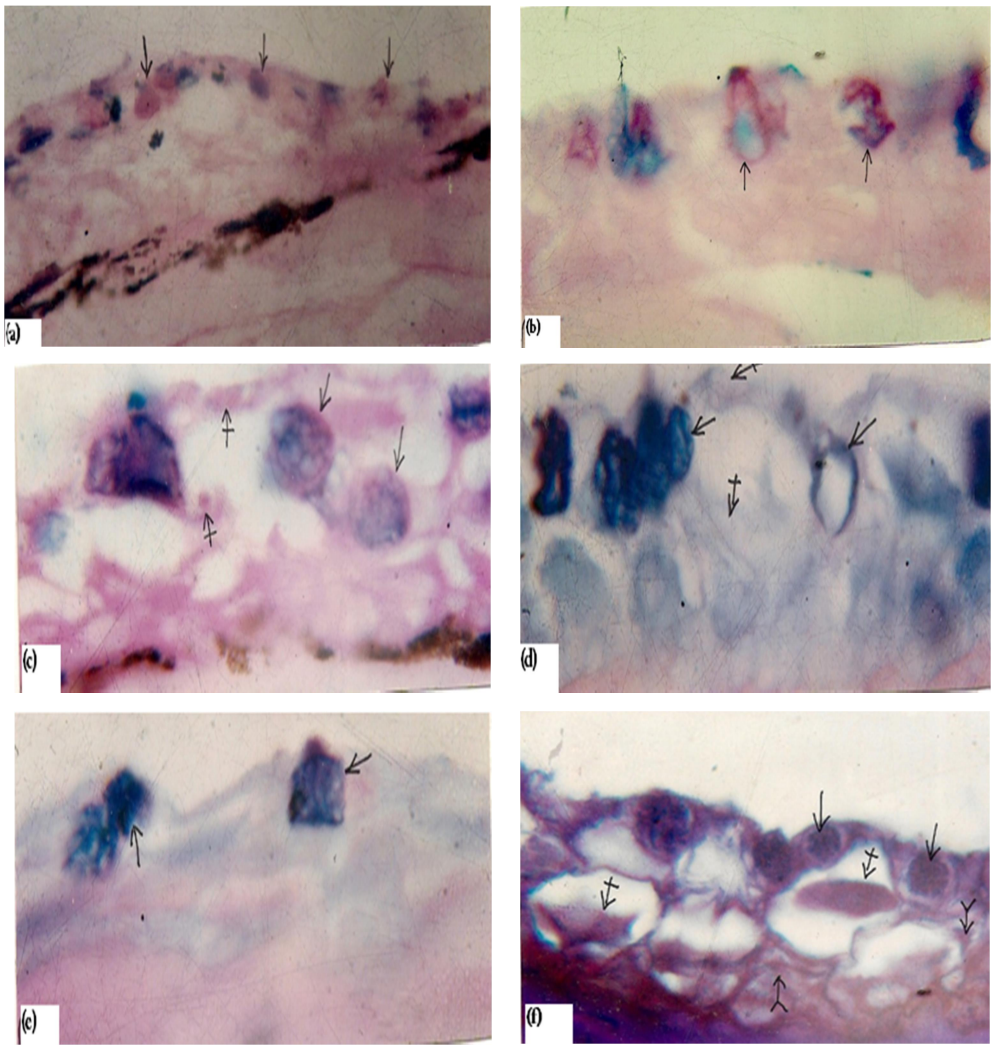

PLATE II (a-f)- Photomicrographs of the cross section of the epidermis of *Ompak bimaculatus* at control and at different duration of the cadmium chloride treatment.

(a) Showing flattening in mucous cells with strong reaction for neutral glycoprotein (arrow) (Sub-lethal, 3h, AB/PAS) x 600.

(b) Showing elongation of mucous cells acquiring flask shape (arrow) (Sub-lethal, 1d, AB/PAS) x 600.

(c) Showing rounded voluminous mucous cells with strong purplish (arrow) and disintegrated epithelial cells staining magenta (barred arrow) (Sub-lethal, 3d, AB/PAS) x 1000.

(d) Showing large acidic glycoprotein containing mucous cells (arrow). Note same reaction in epithelial cells (barred arrow). (Sub-lethal, 5d, AB/PAS) x 600.

(e) Showing few mucous cells with acidic glycoprotein moieties (arrow) (Sub-lethal, 6d, AB/PAS) x 600.

(f) Showing strongly stained acidophil cells (arrow). Note relatively strong reaction in club cells (barred arrow) and epithelial cells (winged arrow) as compared to those in control (Lethal, 3h, MBPB) x 1000.

dimension of mucous cells at 4h, 8h, 24h, 48h, 72h and decrease in mucous cell dimension at 36h and 60h in the epidermis of *Clarias batrachus* exposed to sodium dodecyl sulfate. Phola-Gubo and Adam (1982) in the epidermis of rainbow trout *Salmo gairdeneri* exposed to sodium alkyl benzene sulphonate reported that the mucous cells distinctly increase in number after 24h Zaccone et al.(1985) reported an increase in the number of mucous cells in the epidermis of a catfish *Heteropneustes fossilis* exposed to sodium alkyl benzene sulphonate at 24h and 48h.These authors have however not done statistical analysis to substantiate their observations or take into account the dimensions of mucous cells at different stages of treatment. Roy(1988) reported sudden increase in the number of mucous cells at 45 minutes of treatment in the epidermis of *Rita rita* exposed to dodecyl benzene sodium sulphonate. Maurya et al (2006) have also observed cyclic increase and decrease in density and dimension of mucous cells in the epidermis of *Mystus vittatus* under the influence of different concentration of chrome black T.

Enhanced mucous secretion by the mucous cells forming thick slimy coat over the surface of the epidermis at different duration of cadmium chloride treatment is of great importance. At different exposures wear and tear in surface layer epithelial cells is observed at many sites which may provide invasion sites for various pathogens. The additional amount of mucous covering the damaged sites of the surface layer provides an uninterrupted protective slimy coating preventing entry of pathogens through the damaged sites of the surface layer. Irrespective of class of glycoprotein moieties the role of mucous was like wise postulated previously to inhibit the invasion and proliferation of pathogenic microorganisms and to prevent their colonization in the fish epidermis (Hildemann, 1962; Liguori et al.1963). Further mucous cells have been reported to show agglutinating properties to erythrocytes (Harrisand Hunt, 1973) and haemaglutinic and haemolytic activities (Suzuki, 1985).

In the epidermis of *Ompok bimaculatus* the club cells at different duration of treatment showed enlargement in size secreting their contents into intercellular spaces. At exposures where superficial layer cells were lost as exfoliation, the club cells with broken cell membrane also release their contents to the surface.

At electron microscopic level , appearance of disrupted plasma membrane of club cells and adjacent epithelial cells intact and continuous presence of fibrilar material; structurally similar to that found in the cytoplasm of club cells. Herikson and Matoltsy(1968) to suggest likelyhood the release of the materials from the club cells into intercellular spaces without complete disruption of the epidermis. This is in support of present contention that the club cells release their contents into intercellular spaces in between epithelial cells. The presence of certain number of vesicles and channels which can be occasionally seen opening into intercellular space (Whitear and Mittal, 1983) may further be regarded as an evidence in support of this view. During the experiments on the healing of cutaneous wound in *Rita rita* (Mittal and Munshi, 1974) and hyper osmotic stress to *Barbus sophor* (Agarwal et al, 1979) the club cells remain confined within the epidermis and as a reaction to traumatic condition s at various intervals during the experiment show variable degree of vacuolization and attenuation and subsequently almost disappear almost completely.

The precise role played by the club cells is still matter of great debate. However the protective role played by these protein rich cells against the stress of various physical as well as chemical hazards has been well accepted (Banerjee and Mittal, 1976; Rajan and Banerjee 1991; Chandra and Banerjee 2003).

It is possible that the contents of the club cells released into intercellular spaces may plug the intercellular channels preventing indiscriminate entry of the fluids in the environment surrounding the fish that might be initiated due to disruption of superficial layer of the epidermis under influence of cadmium chloride and thus assisting the fish to cope with the stress. In the late exposure periods the contents of the club cells along with the sloughed epithelial cells form a thick layer on the surface of the epidermis, which may provide a protective barrier layer at certain stages.

4. Conclusion

Toxicity and histopathological studies are useful tools to evaluate pollution potential of a toxicant. Therefore, the substantiation of pathological alternations in organ sequentially in contact with toxicants seems useful as a bimaker of pollutant exposure and effect. This research paper is hoped to provide baseline information of the silence crisis faced by natural environment due to uncontrolled use of heavy metals which finally reach the aquatic habitat in fish farming system. This investigation emphasized the usefulness of the fish epidermal cells as an experimental model for studying the effects of cadmium chloride to the fish.

Acknowledgements

The author is thankful to Principal of College to for the facilities provided to carry out this work, also thankful to colleagues and non-teaching staff for their cooperation.

Conflicts of Interest

The author declares that there are no conflicts of interest.

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