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Arsenic Toxicity on Mucous Cells in the Epidermis of Fresh Water Fish *Catla catla*

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ABSTRACT

Effect of lethal concentration of arsenic has been studied on mucous cells in the epidermis of *Catla catla* in untreated fish generally mucous cells are flask shaped widely separated from each other opening to the surface to void their secretion. Arsenic is a worldwide spread pollutant in chemical form which extensive uses by human in various activities. *Catla catla* is an indian major carp which used for various toxicological experiments in life science and medicine field to be consumed major food sources and highly rich proteins. Due to increasing level of arsenic concentration in ground water considerable attention has been given to the study of effect of arsenic on fish *Catla catla.* Histochemical and histological parameters were used during research work. At 6 hour treatment crowding of mucous cells is observed in entire epidermis. Mucous cells showed increased in dimension; appear vacuolated and closely approximated with each other. At 12 hour decline in density and dimension of mucous cells was observed. At 1day (d), mucous cells showed enormous increase in size. At 2d, mucous cells showed sharp decline in their density and dimension. At 3d, mucous cells showed increase in number and size. At 4d, mucous cells showed decline in number and enormous increase in size. At 5d, mucous cells acquire more dimension as compared to 4d. At 6d, mucous cells got constricted due to secretion of their contents to the surface. At 7d, mucous cells showed decline in number. At 8d, mucous cells showed increase in density occupying whole thickness of the epidermis. At 9d, mucous cells showed decline in density and dimension and looked empty. At 10d, mucous cells appeared enlarged and looked empty. Increase in mucous cell density is due to formation of new mucous cells from basal layer which move upward occupying the different layers of the epidermis and ultimately come to the surface to void copious amount of mucous to cope with altered environmental condition. The mucous cells after secreting mucous evacuated result in their exhaustion and subsequent elimination causing decrease in density of mucous cells. Increase in volume of mucous cells has been associated with increased rate of synthesis of secretory products. The slimy coat over damaged epithelial surface at different duration of treatment has been correlated to prevent entry of pathogens through damaged sites of surface layer which provide invasion sites for micro-organisms.

1. Introduction

Catla catla (Ham.1822) is a surface and midwater carnivorous fresh water fish. It belongs to family Cyprinidae and order cypriniformes that grow to 1-38.6 kg in weight (Froese et al., 2017) at water temperature between 25-32 ^{o}C. Impact of stress inducers on aquatic fauna must be measured (Kumar and Singh 2010). Arsenic and its compounds, especially the trioxide are used in production of pesticides, extensively used as biocide in the aquaculture industry worldwide, is readily absorbed , distributed and accumulated in different tissues and organs in fish and are feared to have adverse effect on fish (Srivastava et al., 2004). Many workers have reported the toxic effects of arsenic on different organ systems of fishes (Srivastava et al., 2004, Patil et al, 2014). Even though the skin epidermis of fish comes in direct contact of ambient water and thus is exposed directly to hazardous chemicals present in polluted water bodies, almost no data is available on the toxicity of arsenic on this vital organ system of *Catla catla.* In this context the present study is an attempt to elucidate toxicopathological effect of arsenic on the mucous cells of skin epidermis of *Catla catla.*

2. Materials and Methods

Specimens of *Catla catla* (length 9.0+1.0 cm; weight 18.0+5.0 g) were collected from local ponds at Singramau, Jaunpur (UP), India during October to January (2019) and were

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acclimatized with laboratory conditions for 15 days before experiments were set. They were fed on every alternate day with minced goat liver. Water was renewed after 24 hours leaving no fecal matter or uneaten food. Arsenic was chosen in the present study because today large quantities of arsenic waste are disposed into aquatic system through agriculture and industrial activities; chemicals used were of analytical grade, purchased from Merck (Varanasi, India). A stock solution of Arsenic trioxide (As_2O_3) was prepared by dissolving 1 gram in de ionized water and then diluted with tap water to obtain the desired concentration. Acclimated fish in batches of ten irrespective of their sex were subjected to 0.1456 mg/L of arsenic (10% of the 96h LC_{50} value 1.456 mg/L) .Similar conditions excepting the addition of arsenic were also maintained in control tanks. Feeding was continued throughout the tenure of experiment. Small fragments of skin from dorsolateral body surface were excised from the fish ,rinsed in physiological saline and were fixed in 10% neutral formalin and aqueous Bouin's at 6 hour (6h), 12h, 1day (1d), 2d, 3d, 4d, 5d, 6d, 7d, 8d, 9d and 10d of arsenic treatment. Standard methods of dehydration, clearing and embedding were used. Paraffin sections were cut at 6μm and subjected to Ehrlich's haematoxylin eosin (HE) to study general organization of the skin. Sections were subjected to various histochemical tests for carbohydrates and proteins following Lillie (1954), Gurr (1958) and Pearse (1985).

3. Results and Discussion

The epidermis is stratified and is separated from underlying dermis by thin non-cellular basement membrane. It may be roughly divided into three principal layers- the superficial layer, the middle layer and the basal layer. It consists of epithelial cells. Interspersed between epithelial cells are found mucous cells, sacciform cells and lymphocytes. Generally mucous cells are flask shaped widely separated from each other, opening to the surface to void their secretions by small pores and extending up to middle layer of the epidermis (PLATE Ia). A few round mucous cells may be observed arising from the basal layer.

3.1 Arsenic Treatment:

After transfer of fishes in arsenic solution, mucous cells showed significant changes in their number, dimensions, shape and histochemical properties at different exposures. At ½ h, the mucous cells acquired voluminous round shaped. In general, they are closely approximated and occupied middle layer of the epidermis. There was gradual increased in the number of mucous cells in the middle layer due to differentiation of new, small and round mucous cells from the basal layer. A very few mucous cells were almost rounded and very small in dimensions, were observed in surface layer of the epidermis. At 1h, the mucous cells of the middle layer move towards surface to void their secretions. They attained larger dimensions and sac like shape. A few vacuoles were observed in mucous cells. The mucous cells were closely approximated. At 2h, the mucous cells, in the surface layer showed overall decline in their dimension due to release of their content at the surface. The middle layer was occupied by almost round mucous cells. A few round mucous cells were also seen arising from the basal layer. At 4h and 6h, crowding of mucous cells was observed in entire epidermis. Mucous cells showed increased in dimension closely approximated with each other actively secreting copious amount of mucous on the surface by wide openings. Some mucous cells were flask shaped, some of dumbbell shaped. A few mucous cells were rounded in shape and smaller in dimension and observed arising from the basal layer. The mucous cells looked vacuolated (PLATE Ib). At 12h, decline in number and size of mucous cells was observed. Mucous cells were widely separated from each other extending up to outer part of the middle layer of the epidermis.

At 1d, no mucous cells were noticed arising from basal layer. The mucous cells show enormous increased in size arranged in one layer, closely approximated with each other and occupy whole width of the epidermis. They were elongated, sac like and vacuolated (PLATE Ic). At 2d, mucous cells showed sharp decline in their density and dimension due to release of their secretory contents to the surface. They were mostly round in shaped. New rounded mucous cells were discernible in the middle layer (PLATE Id). At 3d, mucous cells showed increased in number and size. They were almost round in shaped occupying middle and outer most layers (PLATE Ie). At 4d, the mucous cells showed decline in number and enormous increased in dimension. They were sac like, arranged in a row, opening to the surface, closely approximated with each other due to large dimensions. Fine vacuoles were discernible in these gland cells (PLATE If). At 5d, mucous cells acquired more dimensions as compared to 4d treatment. Heavy vacuolization was observed in these cells (PLATE IIa). At 6d, the mucous cells though elongated, got constricted due to secretion of their contents to the surface. Large vacuoles were observed (PLATE IIb). At 7d, mucous cells showed decline in density, acquired almost oval shape occupying middle layer of the epidermis. No new mucous cells were discernible. At 8d, the mucous cells showed increased in density occupying whole thickness of the epidermis. They showed great variation in their size and shape. Small rounded mucous cells were discernible in the surface and basal layers while large mucous cells were observed in middle layer. The mucous cells were closely approximated due to their large number and dimension (PLATE IIc). At 9d, mucous cells showed sharp decline in size and density. They were mostly oval in shape arranged in three to four layers occupying whole thickness of the epidermis. They looked empty due to release of their secretory contents to the surface and into inter cellular spaces caused by degeneration of the epithelial cells (PLATE IId). At 10d, the mucous cells showed great enlargement in their dimensions. They released most of their contents to the surface and into intercellular spaces. They appeared empty due to release most of their contents (PLATE II e,f).

In control treatment secretory contents of mucous cells in

fishes showed strong reactions, magenta with PAS (Periodic Acid - Schiff Stain) with and without prior diastase treatments which is abolished by acetylation and restored by deacetylation, greenish blue with AB (Alcian blue) at pH 2.5 and purple with AB/PAS indicating the presence of a mixture of acidic and neutral glycoproteins in high concentrations in subsequent treatments. From ½ h to 3d exposures all the mucous cells stain greenish blue with AB pH 2.5/PAS reflecting presence of sulphated acid glycoproteins. From 3d onward mucous cells displayed reactions for both neutral and acidic glycoproteins. At 9d most of the mucous cells stained for acidic glycoproteins, some stain for mixed glycoproteins while a few for neutral glycoproteins. At 10d treatment dominance of sulphated acidic glycoprotein contents was observed in secretory contents within mucous cells as well as in moieties secreted onto the surface and into intercellular spaces. A few mucous cells displayed moderately for mixed glycoproteins.

Significant increase in the dimensions of the mucous cells in the epidermis of *Catla catla* is interesting. It seems that the enlargement of mucous cells at 2h, 6h, 1d, 3d, 4d, 5d, 6d, 8d and 10d of the treatment is caused by the increased rate of synthesis of secretory products within the mucous cells. In the opercular epidermis of the catfish *Rita rita* exposed to the dodecylbenzene sodium sulfonate, Roy (1988) reported that the mucous cells showed a decrease in their diameter throughout the experiment and an increase in their diameter is observed only at 3h. In the gill epithelium of *Catla catla* exposed to a carpet dye, chrome black-T has reported increase in volume and density of mucous cells at different duration of treatment (Mishra et al., 2014).

Increased in number and dimension of mucous cells in the fishes exposed to the arsenic treatment in present investigation may be correlated with increased mucous production. Mucous hyper secretion after exposure to different toxicants has been reported frequently (Burton et al, 1972; Dalela et al, 1997; Garg et al. 1993; Paul et al.,1995; Mishra et al., 2004).

Enhanced mucous secretion by the mucous cells forming a thick slimy coat over the surface of the epidermis in *Catla catla* at different durations of the arsenic treatment is of great importance. At different durations of arsenic exposure wear and tear in surface layer epithelial cells was 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 coating as slime preventing entry of pathogens through the damaged sites of the surface layer. Irrespective of class of glycoprotein moieties the role of mucous was likewise postulated previously to inhibit the invasion and proliferation of pathogenic microorganisms and to prevent their colonization in fish epidermis (Nigrelli et al., 1955; Hildemann, 1962; Lignori et al., 1963). Further mucous has been reported to show agglunating properties to erythrocytes and bacterial antigens and haemaglutinic and haemolytic activities (Suzuki, 1985).

Arillo and Melodia (1990) pointed out that fish mucous has detoxifying action against ambient toxicants. Rajan and Banerjee (1997) opined that mucous helps in retarding the toxicity of the irritant present in the environment. In the gills of *Lepomis macrochirus* exposed to diazonin, Dutta et al. (1997) reported that a mucous film was found over the treated filament and lamellae and several droplets of oozing materials were seen on the cell surface giving the epithelial cells a papullate appearance and this may be a way of eliminating absorbed and metabolized toxicants. Singh et al.(2017) have also observed an increase in density and dimension of mucous cells at gill lamellae of *Channa striatus* exposed to chlordecone. They further observed a thick slimy coat over the lamellae protecting lamellae from noxious effect of the chlordecone.

Oozing of mucous from mucous cells into intercellular spaces resulted due to lysis of epithelial cells is interesting. It is possible that mucous cell contents released into intercellular spaces might serve to plug intercellular channels, preventing discriminate entry of foreign matter that might be initiated due to disruption of superficial layer of the epidermis under influence of arsenic treatment. There is dominance of sulphated acid glycoprotein moieties in the secretory content of mucous cells filling inter cellular spaces and spreading over epidermal surface in *Catla catla*. Sulphation of complex carbohydrates has been shown to result in increased resistance to their enzymatic breakdown by bacterial glycosidases (Mian et al. 1979; Tsai et al.,1992), to play a role in defense against pathogens (Solanki and Benjamin,1982) and to prevent proliferation of pathogenic microorganisms (Tsukise et al., 1981; Suprasert et al.,1986, 1987). The sulphated glycoproteins have also the property of binding with mercury by establishing strong covalent bond with –SH groups of proteins, SS containing amino acids and a wide range of biological molecule (Friberg et al.,1979. Thus high proportions of sulphated glycoproteins in the mucous cell secretions on the epidermal surface and into intercellular spaces under influence of arsenic in *Catla catla* may confer high resistance against pathogens and protected the fish.

Acid glycoproteins have also been shown to coincide with increased viscosity of mucous in the alimentary tract of *Arrhamphus sclerolepis krefftii* (Tibbetts, 1997), in airway epithelia of mammals (Jones et al., 1973; Iravani et al., 1974) and in corals (Miekle et al., 1988). The elaboration of mainly sulphated glycoproteins by mucous cells in late exposure of arsenic in *Catla catla* could thus be related to increase viscosity of mucous and lubrication of the surface of the fish. This could play a vital role in providing protection to the body against mechanical damage during the late exposure period.

The result of present studies will give insight in the field of aquatic toxicology. It is important to determine the lethal concentration of the substance before releasing them into the aquatic environment.

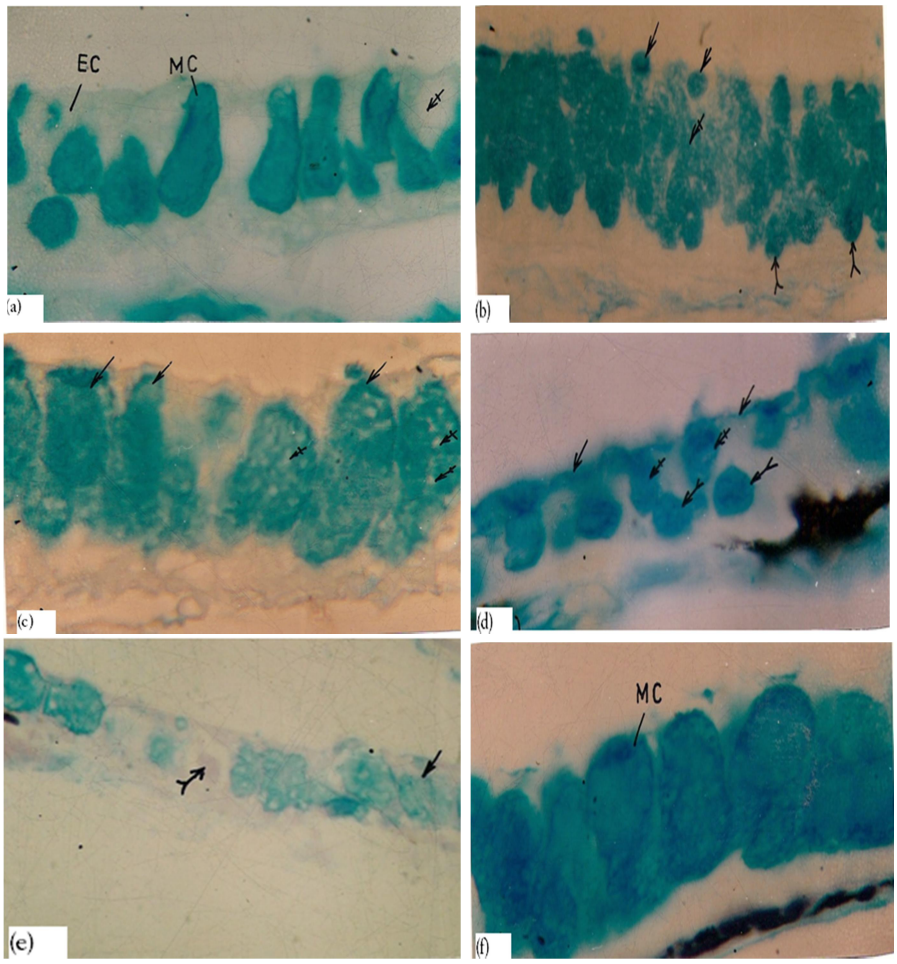

Plate I- (a) to (f)- (a) Photomicrographs of the cross section of the epidermis of *Catla catla* at control and at different duration of arsenic treatment. Showing flask shaped alcian blue positive mucous cells opening to the surface and a few round mucous cells (arrow) above the basal layer. Note moderate greenish blue reaction in epithelial cells and unstained sacciform cells (barred arrow) (AB2.5) x 1000. (b) Showing increase in density of alcian blue positive mucous cells distributed in entire epidermis. Note a few small, round mucous cell (arrow) in the surface layer, some large sized, flask shaped, elongated mucous cells secreting to the surface (barred arrow) and some round mucous cells (winged arrow) arising from the basal layer. (AB 2.5; 6h) x 600. (c) Showing enormously increased mucous cells, arranged in a row closely approximated with each other (arrow). Note fine vacuoles (barred arrow) in the secretory contents of the mucous BM, basement membrane. (d) Showing sharp decline in the density and dimensions of the mucous cells. The mucous cells in the upper layer (barred arrow) get diminish in dimension due to release of most of its contents (arrow) to the surface. Note a second row of mucous cells (barred arrow) underlying the upper layer mucous cells (AB 2.5; 2d) x 1000. (e) Showing the mucous cells of the second tier of 2d treatment reaching to the surface (arrow) to void their secretions. Underlying these cells a second tier of newly formed mucous cells (barred arrow) are observed. Note a sacciform cell (winged arrow) staining purplish. (AB/PAS; 3d) x 600. (f) Showing enormously increased, sac like closely approximated, aclian blue positive mucous cells arranged in a row and opening to the surface to void their secretions. (AB 2.5; 4D) x 1000. MC, mucus cells.

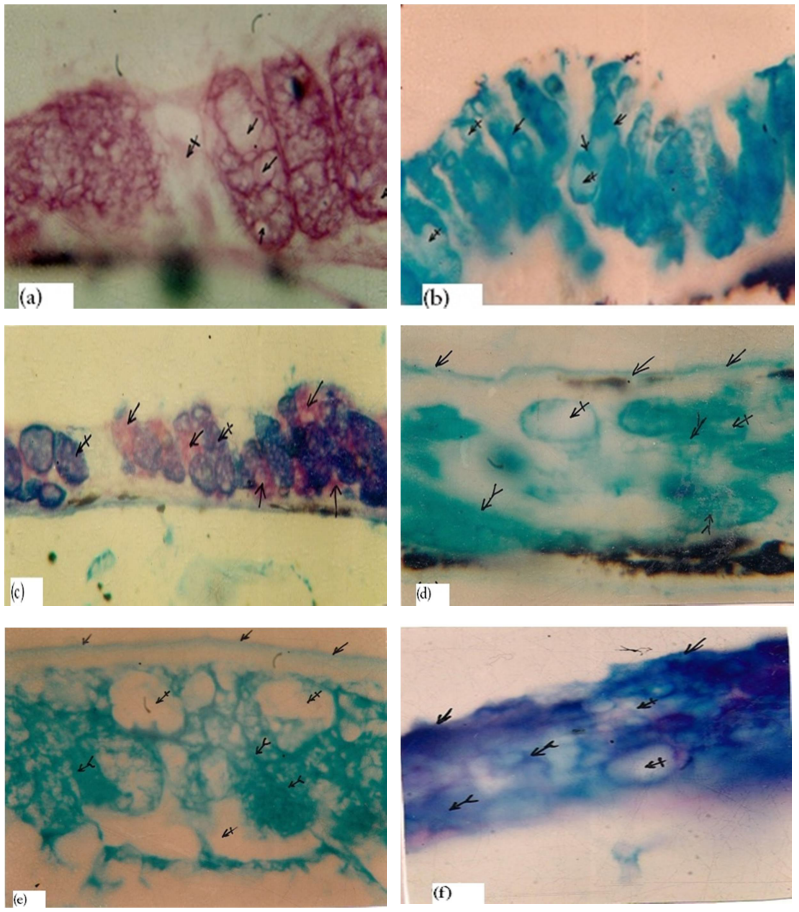

Plate II- (a) to (f)- (a) Photomicrographs of the cross sections of the epidermis of *Catla catla* at different durations of arsenic treatment. Showing quite enlarged mucous cells extending from surface to basement membrane. Heavy vacuolization (arrow) and decline in intensity for neutral glycoprotein is observed in this cell. Noted moderately PAS positive sacciform cell (barred arrow) (PAS; 5d) x 1000. (b) Showing elongated mucous cells with constrictions (arrow) and large vacuoles (barred arrow). (AB 2.5; 6d) x 1000. (c) Showing mixed population of mucous cells. Note mucous cells stained magenta (arrow) and purplish (barred arrow). The epithelial cells stain moderate purplish (AB/PAS; 8d) x 600. (d) Showing evacuation (barred arrow) of mucous cells releasing its contents on the surface (arrow) and into inter cellular spaces (winged arrow). (AB 2.5; 9d) x 1000. (e) Showing alcian blue positive slimy coat over the epidermis (arrow), evacuation (barred arrow) of enlarged mucous cells and release of their acid glycoprotein content into intercellular spaces (winger arrow) (AB; 10d)x 1000. (f) Showing thick mucus coat containing mixed glycoproteins at the surface (arrow), glycoprotein contents into intercellular spaces (winged arrow) and evacuated mucous cells (barred arrow) (AB/PAS; 10d)x1000.

4. Conclusion

The environmental monitoring programmes were evolved to measure impact of stress inducers on aquatic fauna. Aquatic ecosystem is too complex and hence indicators are an efficient means to obtain useful and representative information about the condition of fauna and flora. Acute toxicity studies are very first step in determining the water quality requirement of fish. Epidermis of fish can be helpful to identify that target organ toxic effects and also the general health condition of harmful changes in stressed organisms. The findings of the present study reflect that arsenic exposure of *Catla catla* affect its epidermis. Results of study indicate that arsenic affects the cellular constituents of epidermis in *Catla catla*. Since, *Catla catla* is known as a hardy fish, can tolerate pollutants to a greater extend. If arsenic affects this fish, the extent of damage it may cause to the sensitive fishes and aquatic to terrestrial food web such as planktons to vertebrates will be more serious. Hence the use of arsenic should be avoided or reduced to an extent that our future generations should be protected from the deleterious effect of arsenic.

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Conflicts of interest

The authors declare that there are no conflicts of interest.

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