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HISTOLOGICAL ALTERATIONS IN GILL TISSUES OF *ANABAS TESTIDUNEUS* ON EXPOSURE TO HEAVY METAL $CdCl_2$

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ABSTRACT: Histological alterations are the variations arising in the tissues of the organisms after exposure to certain chemicals found in the ecosystem. These alterations may be in any part or organs of the organisms and have been studied extensively. Biological systems are open to heavy metal exposure in the environment. Various researchers have found cellular and genetic changes in the tissues of organisms more specifically on fishes. Cadmium is toxic to animals which enters surface water from various sources. Being reactive it imparts acute and chronic poisoning. Fishes survive in close interaction with the water through their gills and thus susceptible to heavy metals drained from various sources. In the current study an attempt has been made to assess the impact of $CdCl_2$ on the gill tissues of *Anabas testudineus.* The structural changes in the tissues were noticed. Sub lethal concentration of $CdCl_2$ could disturb growth rate and reproduction causing community disturbances in the tropic levels of food chains. Further, computational genoproteomic studies may shed more light on the general ecophysiology of the fishes.

KEYWORDS: Histology, fish, gill tissues, heavy metal, $CdCl_2$.

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1. INTRODUCTION

Histological alterations are the unwanted changes found in the tissues of animals after exposure towards certain heavy metals. Due to their toxicity, accumulation and biomagnification in water, sediment, and in aquatic food chain [1] along with their association with various diseases [2], these heavy metals leads to significant environmental hazards for aquatic bodies. Fishes being an important source of food are of interest because these are rich in vitamins, calcium, phosphorous

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and iodine [3] and are considered as a good indicator for heavy metal contamination because they occupy different trophic levels; are of different sizes and ages as compared to invertebrates and are also more sensitive to many toxicants [4, 5, 6]. Gills of fishes are the body parts for gaseous exchange and accomplish osmoregulation, acid-base balance and nitrogenous waste excretion [7, 8]. The continuing increase of toxic materials more specifically heavy metals in water due to run off from industries and agriculture have serious impact on the aquatic animals [9]. Thus, the studies on the accumulation of heavy metals in various organs of the fish help in determining the extent of pollution and their causative harmful effects [6, 10]. In the current study an attempt has been made to assess the impact of $CdCl_2$ on the gill tissues of *Anabas testudineus.*

2. MATERIALS AND METHODS

Study area

The present study was carried out in Ganjam District of Odisha, India which extends over an area of 8070.6 square kilometer having a population of 3,520,151 and is located between latitude 19.5860N to longitude 85.051544E with an elevation of around 3 meter (Fig.1). The rivers Ruskuliya, Dhanei, Ghodahada, Bahuda are the prime sources of agriculture. The Chilika lake of Ganjam district is known for its scenic beauty and marvelous bird sanctuary. Gopalpur is a famous commercial port in this district.

Fig 1: Map of Ganjam District: The study area of current research work

Sample collection

For the current study, live and healthy *Anabas testudineus* of uniform size were collected from the non-polluted area of the Ganjam district along with the water samples. The fishes were reared and maintained in the laboratory condition in the decholrinated tap water and no diet was given to them (Fig. 2).

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Fig 2: *Anabas testudineus*: Sample fish species for present research work

Exposure to heavy metal

The fishes were categorized into two groups; one group contained the normal fish that is the control group fishes whereas the other group contained the treated fishes. The treated group was exposed to the heavy metal cadmium chloride ($CdCl_2$) and its LC_{50} value was calculated which was found 191.49 ppm for 96 hour exposure. Then the fishes were exposed to 25% of LC_{50} value of $CdCl_2$ for 24hours for the study of histological alterations found in them.

Histological preparations

After 24 hrs of exposure to heavy metal $CdCl_2$, the gill samples of both control and treated group of fishes were excised, rinsed with deionized water and kept for preservation by using 10% neutral buffered formalin as the compound fixative for histological processing. After 24hours the samples were washed under tap water and the tissues were preserved in 70% alcohol. The tissues were dehydrated by using 90% and 100% alcohols. The tissues were kept in xylene for few minutes for clearing and then transferred to the mixture of xylene and paraffin wax for about 30minutes. During the hot infiltration the tissues were soaked in molten wax for impregnation at a standard temperature coinciding with the melting point of the embedding paraffin wax medium used. This was achieved by passing of the cleared tissue through changes of paraffin wax molten at coinciding melting temperature of wax in each case. The final processing stage was the embedding of tissues in paraffin wax which was necessary to hold the tissue in position and ensure that tissues were not crumbled during sectioning. Blocks were prepared and kept overnight. Then the tissues were trimmed and sections were made using a microtome. For the successful attachment of tissue section along with wax ribbon the cleaned slides were rubbed with bovine serum albumin which was used as adhesive and then the sections were placed on those slides, kept on hot plate for stretching. After that the slides were dried and kept overnight so that those will ready for staining. Sections were deparaffinized in xylene for about 20 minutes and treated with different grades of alcohol i.e. 100%, 90%, 70%, 50% and 30% respectively. The slides were dipped in water, stained in haematoxylin, washed under running tap water, dehydrated via graded alcohol to 90%, counterstained in alcoholic eosin, rinsed in 90% alcohol, dehydrated in absolute alcohol, cleared in xylene and mounted in DPX.

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3. RESULTS AND DISCUSSION

Examination of thin sections of gill arch of *Anabas testudineus* (control) showed four pairs of typical gill arches bearing two rows of primary gill filaments. Each gill filament bears series of alternately arranged semicircular secondary lamellae on both sides. The surface of gill lamella was lined by a thin layer of simple squamous epithelium which rests on basement membrane covering the pillar cell-blood channel system and which constitutes the main vascular area of the gill. There are several reports on the types of histological changes in fish gills due to contaminated water, in field and after acute or chronic exposure in laboratory conditions with sub lethal and lethal concentration of heavy metals like copper, chromium, mercury, cadmium, arsenic, lead, nickel, iron etc. [11-20]. In the present work, gills of *Anabas testudineus* exposed to cadmium chloride solution exhibited varying degree of damage in sub lethal concentrations. Mucus cell hyperplasia was generally more pronounced towards the proximal end of the filament. After 24 hrs of exposure, hyperplasia of epithelial cells resulted in the fusion of many lamellae. The control group of fishes contain normal gill lamella, gill bar and epithelial cell which were clearly seen whereas in treated group fishes the gill tissue were greatly affected like necrosis took place, hyperplasia has been noticed which has perhaps damaged the gill lamella (Fig. 3 & 4).

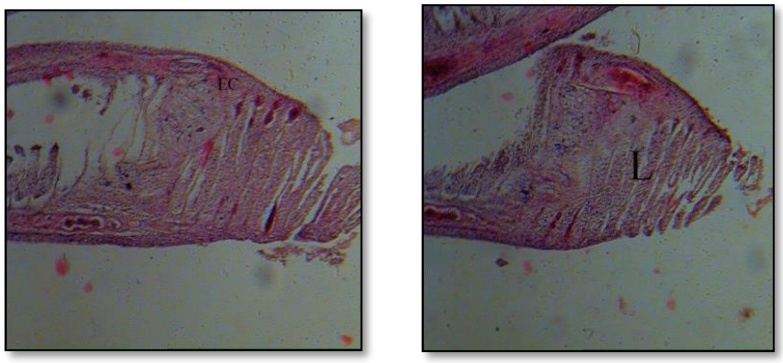

Fig 3: Gill of *Anabas testudineus* (control); EC: endothelial cell, L: lamella

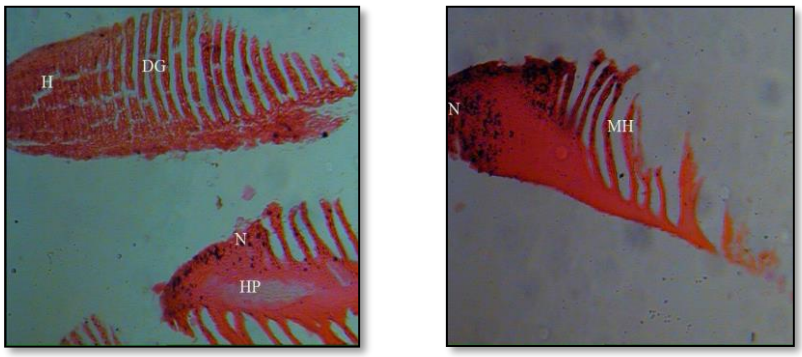

Fig 4: Gill of *Anabas testudineus* (treated); N: necrosis, HP: hyperplasia, MH: mild hyperplasia at base of primary and secondary gill lamella, DG: destroy of gill lamella

Gills have widespread surface area, blood capillaries for efficient gaseous exchange and provided with mucus cells [21]. The mucous discharge works against toxic substances. Due to heavy metal

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intoxication the gill epithelium was completely separated from the basement membrane and pillar cells and there was a swelling of the secondary lamellae and dilation of the vessels. The pillar cell nucleus showed necrosis and vacuolation in the secondary gill epithelium. The disorganized fusion in secondary gill epithelium was prominently noticed. Similar histological alterations in the gills were noticed by Velmurugan and coworkers [22] after exposure to organophosphates leading to epithelial proliferation, congestion of blood vessel and hyperplasia of mucus cells. The physical changes in the gills have been studied [9] leading to necrosis, rupture of the branchial epithelium, autolysis, swelling and lamellar fusion. The accumulations of the heavy metals decrease ventilation which ultimately decreased the O_2 uptake. Similar finding was reported by Prashanth and others [23] leading to epithelial lifting in the Nile tilapia (*Oreochromis niloticus)* under exposure to glyphosate for 96h. The enlargement of chloride secreting cells and their nuclei supports the above assumption. One of the important observations in the present study was the fusion of secondary lamella. This could be attributed to counter stress and transformation of electrically charged properties of the epithelial cells which favor adhesion between the cells of two neighboring secondary lamellae [21]. The fusion of secondary lamellae causing a drastic reduction in the respiratory surface area. Heavy metal could have induced fusion of secondary lamella of gills. Hence it could be assumed that copper sulphate intoxication caused severe aerobic stress in *Anabastes tudineus* leading to wear and tear in the gill epithelium [24].The other variations in the gill epithelium were the separation of respiratory epithelium from basement membrane leading to increasing thickness of secondary lamella thereby decreased diffusion capacity and forming a barrier to prevent entering of dissolved heavy metals. Identical lifting of the respiratory epithelium of the secondary lamella of the gills has also been observed in *H. fossilis* subjected to desiccation stress and lead nitrate exposure [25].

4. CONCLUSION

Cadmium causes toxic effects on gill tissues of fishes leading to structural deformations. In sub lethal concentration it may be fatal for the organisms affecting the growth rate and reproduction resulting in disturbance to whole community and also trophic levels of food chains, ultimately the ecosystem. Further, computational genoproteomic studies may shed more light on the general ecophysiology of the fishes.

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CONFLICT OF INTEREST

There are no conflicts of interests.

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REFERENCES

1. Adeogun AO. Impact of industrial effluent on water quality and gill pathology of *Clarias gariepinus* from Alaro stream, Ibadan, Southwest, Nigeria. European Journal of Scientific Research. 2012; 76 (1): 83-94.
2. Uwem GU, Emile AF, Udo IJ, et al. Bioaccumulation of heavy metal in three fresh water fishes caught from cross river system. European Journal of Experimental Biology. 2013; 3(3): 576-582.
3. Zaki MS, Shalaby SI, Ata N, et al. Effect of Aquatic Pollution on Fish (Review). Life science journal. 2013; 10(1): 637-642.
4. Powers DA. Fish as model systems. Science. 1989; 246(4928): 352-3588.
5. Wester PW, Vethaak AD, van Muiswinkel WB. Fish as biomarkers in immunotoxicology. Toxicology. 1994; 86(3): 213-232.
6. Yancheva V, Velcheva I, Stoyanova S, et al. Histological Biomarkers In fish as a tool, Ecological risk assessment and monitoring programs: A Review. Applied Ecology and Environmental Research. 2015; 14(1): 47-75.
7. Heath AG. Water pollution and fish physiology. CRC Press, Florida. 1987.
8. Hadi AA, Alwan SF. Histopathological changes in gills, liver and kidney of fresh water fish, *Tilapia zillii*, exposed to aluminum. International Journal of Pharmacy & Life Sciences. 2012; 3(11): 2071-2081.
9. Dutta HM, Munshi JSD, Roy PK, Singh NK, Adhikari S-Ultrastructural changes in the respiratory lamellae of the catfish *Heteropneustes fossilis* after sub lethal exposure of malathion. Environ Pollut. 1996; 3: 329-341.
10. Shivakumar CK, Thippeswamy B, Tejaswi kumar MV, et al. Bioaccumulation of heavy metals and its effect on organs of edible fishes located in Bhadra River, Karnataka. International Journal of Research in Fisheries and Aquaculture. 2014; 4(2): 90-98.
11. Doughtie DG, Rao KR. Ultrastructural and histological study of degenerative changes leading to black gills in grass shrimp exposed to a dithiocarbamate biocide. Journal of Invertebrate Pathology. 1983; 41 (1): 33-50.
12. Alazemi BM, Lewis JW, Andrews EB. Gill damage in the freshwater fish *Gnathonemus Petersii* (Family: Mormyridae) exposed to selected pollutants: an ultrastructural study. Environmental Technology. 1996; 17 (3): 225-238.
13. Mazon AF, Monteiro EAS, Pinheiro GHD, et al. Hematological and physiological changes induced by short-term exposure to copper in the freshwater fish, *Prochilodus scrofa*. Brazilian Journal of Biology. 2002; 62(4a): 621-631.
14. Rao JV, Shilpanjali D, Kavitha P, et al. Toxic effects of profenofos on tissue acetylcholine esterase and gill morphology in a euryhaline fish, *Oreochromis mossambicus*. Archives of Toxicology. 2003; 77(4): 227-232.

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15. Camargo MMP, Martinez CBR. Histopathology of gills, kidney and liver of a Neotropical fish caged in an urban stream. Neotropical Ichthyology. 2007; 5 (3): 327-336.
16. Matos P, Fontaínhas-Fernandes A, Peixoto F, et al. Biochemical and histological hepatic changes of Nile tilapia *Oreochromis niloticus* exposed to carbaryl. Pesticide Biochemistry and Physiology. 2007; 89(1): 73-80.
17. Velcheva I, Arnaudov A, Georgieva E. Influence of zinc on gill morphology of *Gibelio* carp (*Carassius gibelio*). Ecologia Balkanica. 2010a; 2: 19-23.
18. Velcheva I, Tomova E, Arnaudova D, et al. Morphological investigation on gills and liver of freshwater fish from dam lake "Studen kladenets". Bulgarian Journal of Agricultural Science. 2010b; 16: 364-368.
19. Sousa DBP, Almeida ZS, Carvalho-Neta RNF. Histology biomarkers in two estuarine cat fish species from the Maranhense Coast. Arquivo Brasileiro de Medicina Veterinária e Zootecnia. 2013a; 65(2): 369-376.
20. Sousa DBP, Almeida ZS, Carvalho-Neta RNF. Integrated analysis of two biomarkers in Sciades herzbergii (Ariidae, Siluriformes), to assess the environmental impact at São Marcos? Latin American Journal of Aquatic Research. 2013b; 45: 305-312.
21. Jha JK, Ranjana, Kumar P, Mishra AP- Histopathological changes in the gills of *Channa gachua*, an air breathing teleost after short term exposure of hostathion. Bioscan.2014;9(3): 925-929.
22. Velmurugan B, Selvanayagam M, CengizEI,Unlu E- Histopathological changes in the gill and liver tissues of freshwater fish, *Cirrhinus mrigala* exposed to dichlorvos. Braz. Arch. Biol. Technol. 2009; 52 (5): 1291-1296.
23. Prashanth MS, Sayeswara HA, Goudar MA- Effect of sodium cyanide on behavior and respiratory surveillance in freshwater fish Labeo rohita (Ham). Recent Research in Science and Tech. 2011; 3: 24-30.
24. Kumar SV, Pascal LF, Tennyson S, Pandeeswari M, Dhinamala K, Persis D, Raveen R, Arivoli S, Meeran M-Histopathological studies of *Anabas testudineus* Bloch 1792 on exposure to aquatic toxicants of Buckingham canal, Chennai, Tamil Nadu, India. International J. Biology Res. 2018; 3(2): 125-133.
25. Parashar RS, Banerjee TK- Toxic impact of lethal concentration of lead nitrate on the gills of air breathing catfish *Heteropneustes fossilis* (Bloch). Vet Arhiv. 2002; 72: 167-183.

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