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HISTOLOGICAL ALTERATIONS IN GILL TISSUES OF ANABAS TESTIDUNEUS ON EXPOSURE TO FERTILIZER DAP

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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 fertilizer DAP exposure in the environment. Various researchers have found cellular and genetic changes in the tissues of organisms more specifically on fishes. Fishes survive in close interaction with the water through their gills and thus susceptible to fertilizer drained from various sources. In the current study and attempt has been made to assess the impact of DAP on the gill tissues of Anabas testudineus. The structural changes in the tissues were noticed. Sub lethal concentration of DAP 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.

KEY WORDS: *Histology, fish, gill tissues, Fertilizer, DAP.*

1. INTRODUCTION

Histological alterations are the unwanted changes found in the tissues of animals after exposure towards certain fertilizer DAP. Due to their toxicity, accumulation and biomagnifications in water , sediment, and in aquatio food chain [1] along with their association with various diseases [2], these fertilizer DAP leads to significant environment hazards for aquatic bodies. Fishes being an important source of food are of interest because these are rich in vitamins, calcium, phosphorous and iodine [3] and are considered as a good indicator for fertilizer contamination because they occupy different tropic 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 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 DAP 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 DAP on the gill tissues of Anabas testudineus.

2. MATERIALS AND METHODS

Study area

The present study was carried out in Darbhanga District of Bihar. Darbhanga district is one of the thirty-eight districts of Bihar state in eastern India, and Darbhanga city is the administrative headquarters of this district and 5th largest city of Bihar as well. Darbhanga district is a part of Darbhangfa Division. The district is bounded on the north by Madhubani district, on the south by Samastipur district, on the east by Saharsa district and on the west by Sitamarhi and Muzaffarpur districts.

Sample collection

For the current study, live and healthy Anabas testudineus of uniform size were collected from the non-polluted area of the Darbhanga 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. 1)

Fig 1: Anabas testudineus: Sample fish species for present Reseach work

Exposure to DAP

The fishes were categorized into two groups; one group contained the normal fish that is the contro group fishes whereas the other group contained the treated fishes. The treated group was exposed to the fertilizer DAP for 24 hours for the study of histological alterations found in them. Histological preparations After 24 hrs of exposure to DAP, 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 crimbled 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 rbbon 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, washe 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.

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 alternate ly 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 basemebnt membra ne 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 contaminate d water, in field and after acute or chronic exposure in la boratory conditions with sub lethal and lethal concentration fertilizer like DAP phosphate etc. [11-20]. In the present work, gills of Anabas testudineus exposed to DAP solution exhibited varying degree of damage in sub lethal concentrations. Cell hyperplasia was generally more pronounced towards the proximal end of the filament. After 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 group fishes the gill tissue were greatly affected like necrosis took place, hyperplasia has been noticed which has perhaps damaged the gill lamella (Fig. 2&3).

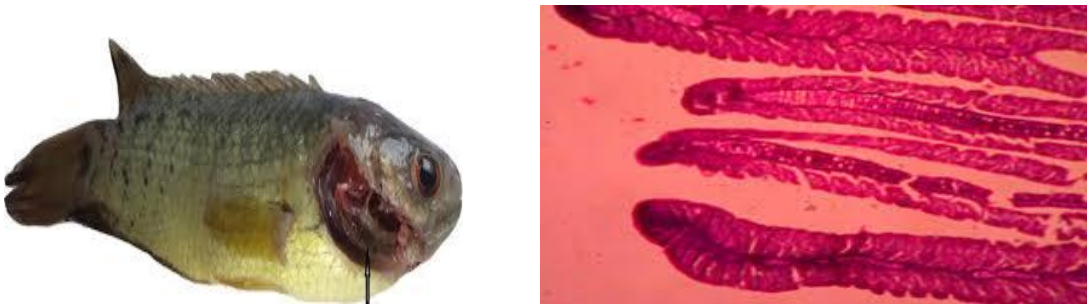

Fig 2. Gill of Anabas testudineus (control); EC : endothelial cell, L: lamella

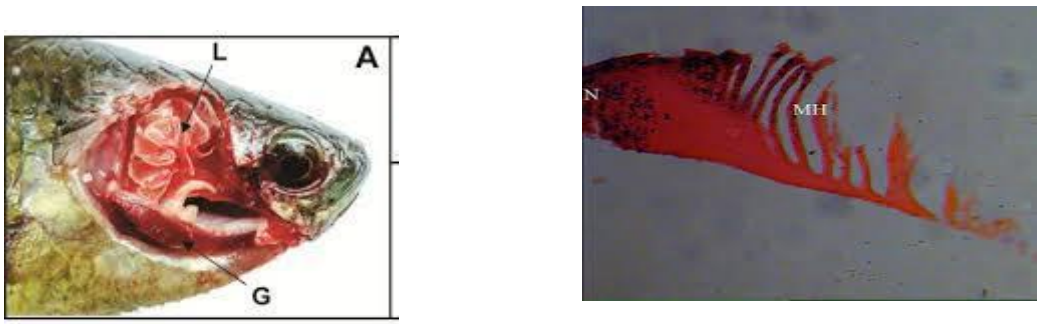

Fig 3: Gill of Anabas testudineus (treated); N: Necrosis, HP: hyperplasia. MH: mild

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 DAP intoxication the gill epithelium was compeletely 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 leadin o epithelial proliferation, congestion of blood vellse and hyperplasia of mucus cells. The physical changes in the gills have been studied leading to necrosis, rupture of the bra nchial epithelium, autolysis, swelling and lamellar fusion. The accumulations of the DAP decrease ventilation which ultimately decreased the O2 uptake. Similar finding was reprte d 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 o counter stress and transformation of electrically charged properties of the epithelial cells which favor adhesion between the cells of two neighboring secondary lamellae. The fusion of secondary lamellae causing a drastic reduction in the respiratory surface area. DAP could have induced fusion of secondary lamella of gills. Hence it could be assumed that copper sulphate intoxication caused severe aerobic stress in Anabas testudineus leading to wear and tear in the gill epithelium [24]. The other variations in the gill epihelium were the separation of respiratory epithelium from basement membrane leading to increasing thickness of secondary lamella t hereby decreased diffusion capacity and forming a barrier to prevent entering of dissolved DAP. Identical lifting of the respiratory epithelium of secondary lamella of the gills has also been observed in H. fossilis subjected to desiccation stress [25].

4. CONCLUSION

DAP causes toxic effects on gill tissues leading to structural deformations. In sub letha l concentration it may be fatal for the organisms affecting the growth rate and reproduction re sulting in disturbance to whole community and also tropic levels of food chains, ultimately the ecosystem . Further, computational genoproteomic studies may shed more light on the general exophysiology of the fishes.

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