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RESEARCH ARTICLE

HISTOPATHOLOGICAL CHANGES IN GILL AND KIDNEY OF *CTENOPHARYNGODON IDELLA* EXPOSED TO ARSENIC TRIOXIDE

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Abstract

Arsenic, a toxic metalloid, is prevalent in the environment, where it occurs both naturally and as result of pollution. In this context, the present work has been proposed to study the effects of sub-lethal concentrations of arsenic trioxide on the histology of gill and kidney of *Ctenopharyngodon idella* after 28 days exposure. The histopathological changes alterations observed in the gills were hyperplasia, lamellar fusion, destruction of the lamellar epithelium, deletion of secondary lamellar and kidney were vacuolization of tubular cells, shrinkage of glomeruli, pycnotic nuclei, disintegration of haemopoietic tissue. In conclusion, gills and kidney of *Ctenopharyngodon idella* showed profound histological alterations as a result of arsenic trioxide exposure, and hence, proved to be excellent indicators of environmental contamination.

Keywords: Arsenic trioxide, *Ctenopharyngodon idella,* Gill & kidney tissues and Histopathology.

Introduction

Environment is the sum total of water, air and land interrelationships among themselves and also with the human being, other living organisms and other property. Environment pollution occurs when the environmental degradation crosses limit so that, it becomes lethal to living organisms. While pollution is always anthropogenic, environmental degradation also includes natural factors (Mathivanan, 1988; Ozden, 2010). Further, environmental pollution is caused by rapid industrialization, modern technologies and informal settlements. Aquatic systems are often disturbed by anthropogenic sources in general and heavy metal pollutants in particular. Thus, there will be a gradual increase in the level of such metals in aquatic environment and has become a problem of primary concern (Bose *et al.,* 1994).

Arsenic, a toxic metalloid, is prevalent in the environment, where it occurs both naturally and as result of pollution. Exposure to arsenic *via,* occupational and environmental sources represents a major health concern worldwide according to the World Health Organization (WHO, 2001). Any change in the natural conditions of aquatic organisms causes several adjustments in fish and metals are the main culprit for these undesirable changes in water quality (Garg *et al.,* 2009). Due to their toxicity, long persistence, bioaccumulative and non-biodegradable properties in the food chain, metals constitute a core group of aquatic pollutants. The contamination of water by arsenicals and consequent toxicity in aquatic organisms has now emerged as a global environmental problem. Recently, anthropogenic activities such as treatment of agricultural land with arsenical pesticides and herbicides, industrial processes such as smelting of other metals, treating of wood using chromatid copper arsenate, burning of coal in thermal plant power stations, power generation from coal or geothermal sources and operations of gold-mining have increased the environmental

pervasiveness of arsenic and its rate of discharge into freshwater habitat (Sprocati *et al.,* 2006).

Arsenic can be found in both organic and inorganic compounds with variable oxidation states. More than one hundred million people are at high risk of elevated arsenic exposure, mainly *via* drinking water as well as by the air born metalloid in the areas with coal burning and industrial emissions. The consumption of the arsenic contaminated fishes collected from the polluted waters might also contribute to bioaccumulation of arsenic in human. Hence, it is of immense importance to know the arsenic induced damages in different organ systems of fishes used for human consumption (Zhou *et al.,* 2008).

The arsenic is graded as one of the most toxic elements to fish. Acute exposure can result in immediate death, because of arsenic induced increases in mucus production, causing suffocation or direct detrimental effects on gill epithelium. Gill surfaces are the first target of water-borne metals (Spicer and Weber, 1991). The gills of aquatic organisms are a primary focus of disturbances caused by the action of pollutants, since they are having indirect contact with the external environment, carrying out their functions of gas exchanges and ion balance (Poleksic and Mitrovic-Tatundzic, 1994). Kidney plays a major role in the elimination of most of the toxicant and considered as a major target organ for toxicity impact (Eva, 1990).

Histopathology deals with the study of pathological changes induced in the microscopic structure of the body tissue. Any peculiar type of alteration of cells may indicate the presence of the disease or the effect of toxic substance. Thus, the study of histopathology is of prime importance in the diagnosis, etiology and prevention of disease. In fishes, it is observed that, the external organs are affected due to toxic chemicals, causing loss of equilibrium, and increase in opercular movements, to and for irregular vertical movements, finally leading to death. This may be attributed to the significant damage to the internal organs. Histopathological study thus gives us useful data concerning tissue change prior to external manifestation.

The histopathological changes produced by heavy metals in different tissue have been described by many investigators (Mathivanan, 1988; Fanta *et al.,* 2003 and Datta *et al*., 2009). The present study has been focused on arsenic trioxide induced histopathological changes of the vital organs like gill and kidney.

Materials and Methods

The fish, *Ctenopharyngodon idella* having mean weight 25 - 30 gm and length 14 – 16 cm were collected from PSP fish farm, at Puthur and acclimatized to laboratory conditions. They were given the treatment of 0.1%$KMNO_4$ solution and then kept in plastic pools for acclimatization for a period of nine days. They were fed on rice bran and oil cake daily. The arsenic trioxide was used in this study and stock solutions were prepared. Arsenic trioxide LC_{50} values were 3.28 ppm taken as sub-lethal concentrations for this study. Twenty fish were selected and divided into 2 groups of 10 each. The first group was maintained in free from arsenic trioxide and served as the control. The second group was exposed to sub-lethal concentration of arsenic trioxide for 28 days exposure period. At the end of each exposure period, the fish were sacrificed and the required tissues were collected for histology and histopathological examination.

Histopathological examination

To examine the extent of cellular damage caused by the Arsenic trioxide in the gill and kidney tissues of fish, *Ctenopharyngodon idella* control and experimental groups were fixed in Bouin's fluid for 24 hours. After 24 hours, the standard histological technique was followed by the method of Gurr (1959). The tissues were dehydrated in graded series of alcohol, cleared in xylol and embedded in paraffin wax (58°-60°C). Using a rotary microtome, 6µ thick sections were cut. The sections were deparaffinized in xylol, passed through descending grades of alcoholic series and then washed with distilled water. Then the sections were stained with Heidenhain's iron haematoxylin and counterstained with aqueous eosin. Stained sections were mounted in DPX for microscopic observations.

Results

Observations

Histology of gill in control fish

The gill of control freshwater fish, *Ctenopharyngodon idella* is characterized by the

presence of primary lamellae along with the secondary lamellae conformity to general architecture design of the tissue. It further showed the mucus cells lying scattered on both the sides of epithelium. The primary lamellae were thicker than the secondary lamellae (Fig.1). The primary lamellae are lined on either side by the multilayered epithelium and closely arranged to the cartilaginous gill ray which runs in the centre of the primary lamellae as a supporting structure. The primary lamellae was broad and bears on either side a series of transverse, flat, leaf like structures called the secondary lamellae. The secondary gill lamellae were covered with flattened epithelial cells attached to the basement membrane, contractile pillar cell system and blood spaces were also present (Fig.1).

Histology of treated fish gill after 28 days

The gill of *Ctenopharyngodon idella* treated with sublethal concentration of arsenic trioxide for 28 days exhibited remarkable changes. The histopathological lesions caused by the arsenic were curling of lamellae and degeneration of the lamellar epithelium, hyperplasia, excessive secretion of mucous in the intercellular spaces, the proximal region of the gill rays get damaged and become blunt and swollen, the respiratory epithelial cells were found destroyed in most secondary lamelleae, shrunken nuclei and vacuoles in the cytoplasm were also observed in the secondary lamellae, reduction in length, swelling of epithelial cells of secondary lamellae, cytoplasmic vacuolization and deletion of the secondary lamellar epithelium (Fig.2).

Histology of kidney at control fish

The kidney tissue observed in Glomerulus and Bowman's capsules and it is made up of epithelial cells. The glomerulus consists of afferent and efferent arterioles, a capillary tuft and visceral and parietal epithelial cells were also formed. Renal tubules consist of proximal distal convoluted tubules. The proximal convoluted tubule was lined by columnar epithelial cells and the distal convoluted tubule was lined by dome shaped cells, as well as basophilic intercalated cells towards the base (Fig.3).

Histology of treated fish kidney after 28 days

The fish *Ctenopharyngodon idella* exposed to sublethal concentration of arsenic trioxide showed remarkable histopathological changes such as necrosis of kidney tubules, vacuolization of tubular cells, shrinkage of glomeruli, pycnotic nuclei, disintegration of haemopoietic tissue, enlargement in the lumen of the tubules and degeneration of renal tubules (Fig.4).

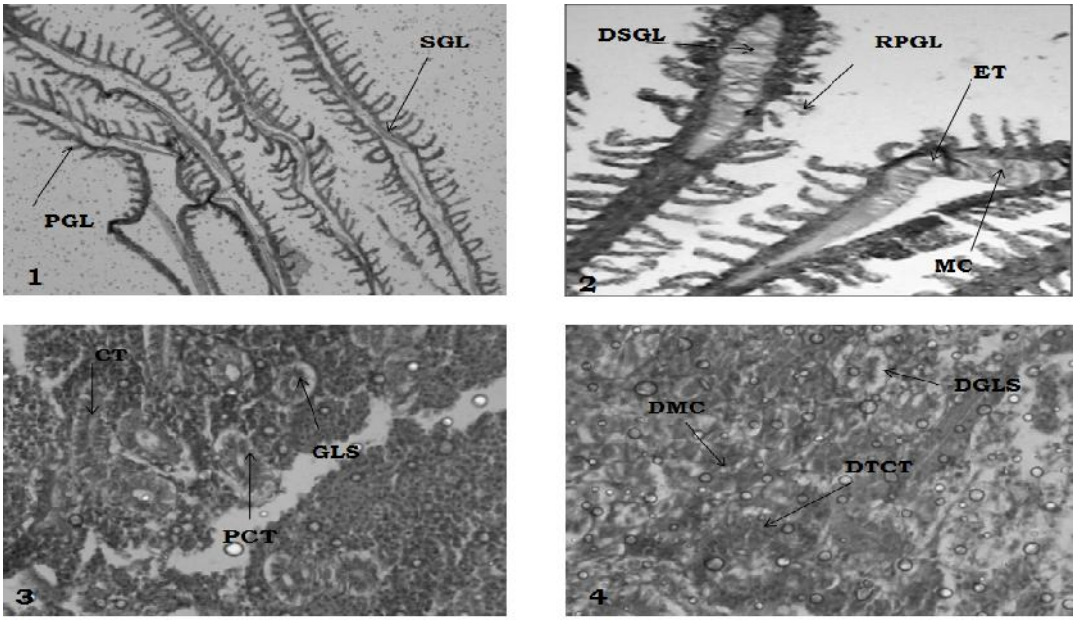

Fig. 1 Control gill; **Fig. 2** Arsenic trioxide treated gill after 28 days; **Fig. 3** Control kidney; **Fig. 4** Arsenic trioxide treated kidney after 28 days.

Fig 1&2. PGL - Primary Gill Lamellae; **SGL** – Secondary Gill Lamellae; **ET-** Eroded Tip; **DPGL** - Disintegrated Primary Gill Lamellae; **DSGL**– Disintegrated Secondary Gill Lamellae; **MC** - Mucous Cells; **IT**- Intra cellular space.

Discussion

The contamination of freshwater with a wide range of pollutants has become a matter of concern over the last few decades (Dirilgen, 2001 and Vutukuru, 2005). The natural aquatic system may extensively be contaminated with heavy metals released from domestic, industrial and other man made activities (Velez and Montoro, 1998). Heavy metal contamination may have devasting effect on the ecological balance of the recipient environment and diversity of aquatic organisms (Vosyliene and Jankiite, 2006; Farombi *et al,* 2007).

In the present investigation, the gill of *Ctenopharyngodon idella* treated with sublethal concentration of arsenic showed curling of lamellae and degeneration of the lamellar epithelium, hyperplasia, and excessive secretion of mucous in the intercellular spaces, reduction in length, swelling of epithelial cells of secondary lamellae, cytoplasmic vacuolization and deletion of the secondary lamellar epithelium. A similar observations have been made in the gills of freshwater fish, *Cyprinus carpio* when exposed to sublethal concentration of arsenic (Kawser Ahmeda, *et al.,* 2013), *Cyprinus carpio* when exposed to sublethal and lethal concentration of fluoride (Jinling Cao *et al.,* 2013), rainbow trout, *Salmo gairdneri* exposed to mercury and copper (Daoust *et al.,* 1984), *Channa punctatus* exposed to various heavy metals (Pandey *et al.,* 2008), *Lisa parsia* exposed to DDT (Pandey *et al.,* 1993). Injuries in gill tissues as reported due to arsenic exposure may reduce the oxygen consumption and disrupt the osmoregulatory function of the fish (Kawser Ahmeda, 2013).

Kapila Manoj and Ragothaman, (1999) have reported severe changes in the histology of gills which led to the disturbance in basement membrane, degeneration of gill lamellae, cyst formation and increased inter lamellar space in *Boleophthalmus dussumieri* exposed to sublethal concentration of cadmium. Marina *et al.* (2007) have observed the cyst formation in the gills after the exposure to toxic metals. A complete damage in the gill of *rainbow trout* was found during nonmaterial's intoxication (Federici *et al.,* 2007). Shrinkage of the respiratory area and enlargement of the water barrier of the gill in the sublethel concentration of the cadmium has been reported in fish, *Lactes calcarifer* (Thophon *et al*., 2003). Metallic mixture produced severe damage to the gill of *Cyprinus carpio* exposed to cadmium and mercury (Dhanapakiam *et al.,* 1998). The secondary lamellae, leading to intense epithelial detachment, destruction and deletion of secondary lamellae, these structural changes were considered the main causes of exposure to heavy metals (Oliveira Ribeiro *et al.,* 2005; Nero *et al.,* 2006).

Kidney is an important organ of excretion and osmoregulation and it is highly susceptible to toxic substances because of it's high blood supply. Kidney plays a major role in the elimination of most of the toxicants and it is considered as a major target organ for toxicity impact (Mathivanan, 1988). Kotsanis and Iliopoulou-Georgudaki (1999) have reported arsenic-induced fibrosis in the kidney of rainbow trout (*Oncorhynchus mykiss*) and histological lesions were observed in lake white fish (*Coregonus clupeaformis*) kidney with dietary arsenic exposure (Pedlar *et al.,* 2002). In the present investigation, when fishes treated with sublethal concentration of arsenic for 28 days showed vacuolization of tubular cells, pycnotic nuclei, shrinkage of glomeruli, enlargement in the lumen of the tubules and degeneration of renal tubules, disintegration of haemopoitic tissue, necrosis of kidney tubules. These findings were similar to those of Datta *et al.* (2009) in *Clarias batrachus* exposed to arsenic, Moorthikumar, (2011) in *Labeo rohita* exposed to nickel chloride, Dhanapakiam and Premlatha, (1994) in *Cyprinus carpio* exposed to malathion after chronic periods, Pandey *et al.* (1997) in *Oreochromis mossambicus* exposed to ammonia after long-term periods and Ravindrababu and Neeraja, 2012) in *Dicentrachus labrax* exposed to mercury. Further, the present findings are in dilation of the lumen of the kidney tubules, degeneration in the heamopoitic tissue rupture in the collecting tubules and necrosis are observed after chloropyrifos treatment have also been reported in various fingerlings exposed pollutants (Vardhani and Gowri, 2002).

Vacuolated cytoplasm and dilation of nuclear envelop were observed in the kidney of *Fundulus heteroclitus* after exposure to mercury Shalaby (2009). The present experimental study, exhibited the presence of vacuoles in the collecting tubules and necrosis of kidney tubules in arsenic exposed fish, *Ctenopharyngodon idella*. A similar observation made in the kidney of *Cyprinus carpio* when exposed to chromium (Parvathi *et al.,* 2011), *Channa punctatus* exposed to heavy metals Pandey *et al.* (2008) and *Channa puntatus* exposed to PMA (Karupasamy, 2000).

The induction of tubular degeneration, coupled with the presence of necrosis in the kidney in our study indicated that the kidneys had suffered detrimental damage induced by the exposure of cadmium. The development of new nephrons in fish continues throughout life (Reimschuessel, 2001). The glomerular shrinkage, vacuolization, pycnosis, cytolysis of the epithelial cells of tubules and complete necrosis of a few proximal convoluted tubules were reported in the mullet*, Lisa parsia* exposed to sublethal concentration of lead under long term exposure (Pandey *et al.,* 1997). Degeneration of renal tubules, disintegration of haemopoietic tissue, formation of vacuoles around glomeruli and edema were found in cadmium, copper and mercury treated fish, *Etroplus suratensis* under 10, 20 and 30 days of exposure (Magendran, 1990). Vacuolated cytoplasm and dilation of nuclear envelop were observed in the kidney of *Fundulus heteroclitus* after exposure to mercury (Shalaby, 2009). The present study in *Ctenopharyngodon idella,* exposed to sublethal concentration of arsenic trioxide fall in line with the observations of earlier investigators (Akter *et al*., 2008; Capkin *et al.,* 2009 and Guardiola *et al.,* 2013).

Conclusion

Exposure with arsenic creates manifold disturbances in the target organs. Disruption of the histology organization in the organ systems and consequent impairment in enzyme activities all contribute to the toxic impact of the arsenic trioxide.

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