INTERNATIONAL JOURNAL OF ADVANCED BIOLOGICAL RESEARCH

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ULTRASTRUCTURAL STUDIES ON THE SUB-LETHAL EFFECTS OF ARSENIC TRIOXIDE ON GILLS OF FRESHWATER FISH, *CTENOPHARYNGODON IDELLUS* (VAL.)

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ABSTRACT
Present investigations suggested that fish are highly susceptible to various concentrations of arsenic trioxide to grass carp, *Ctenopharyngodon idellus* (Valenciennes). Among various organs, gills are badly damaged. Ultrastructural changes in gills have been studied using technique of scanning electron microscopy. Our results on the toxicity of arsenic trioxide on gill architecture of grass carp at two sub- lethal concentrations (5.745 mg/ L ($1/3^{rd}$ of LC_{50}) and 2.462 mg/ L ($1/7^{th}$ of LC_{50}) for a period of 30 days and 60 days respectively reveal various degrees of damage in terms of disruption of microridges increasing with increased dosage and duration, upliftment of epithelial cells and also development of hyperplasia condition along with complete dystrophy of the gill lamellae. Thus, it is observed that arsenic trioxide is harmful for fish health at varying concentrations, so unethical practice of pesticides having arsenic should be avoided.

KEYWORDS: Arsenic, *Ctenopharyngodon idellus*, Gills..

INTRODUCTION

During the last few years, there has been a rapid increase in the use of various pesticides to augment agriculture products. Several heavy metals such as arsenic, cadmium, lead *etc*. have been recklessly used in many modern day pesticides. These heavy metals and pesticides reach the water body with run- off water and persist there in very low concentrations (Tilak *et al.*, 2004). Effect of pesticides and other pollutants in fish can be indicated by using gill tissues as biomarkers (Nowak, 1992; Johal and Dua, 1994) as gills come in direct contact with contaminated water. Gills show symptoms earlier than other vital organs (Richmonds and Dutta, 1989). The large surface area and fine sieve like structure of gills make them highly susceptible to continuous exposure to waterborne noxious agents (Lichtenfels *et al.*, 1996). Due to these reasons, gills are considered good indicator of water quality (Rankin *et al*., 1982), and also perfect model for studies of environmental impact of toxicants (Bonga and Lock, 1991). The present work has been carried out by exposing grass carp, *Ctenopharyngodon idellus* (Valenciennes) to two sub-lethal concentrations of arsenic trioxide *i.e.* 5.745 mg/L ($1/3^{rd}$ of LC_{50}) and 2.462 mg/L ($1/7^{th}$ of LC_{50}) for a period of 30 days and 60 days respectively for studying the ultrastructural changes in the gills. The result of present study will help the environmentalists and policy makers to find out new strategies reducing the level of water pollution.

MATERIALS & METHODS

Live specimens of *Ctenopharyngodon idellus* (Val.) having total fish length of 18.00 cm (±4.00cm) were brought from Neelamber Fish Farm at Nanoke near Nabha, district Patiala, Punjab during the month of October, 2010. The average weight of fish was 20 grams (±5); fish were acclimatized to laboratory conditions for 15 days in glass aquarium provided with filters and aerators. Feeding was done using artificial feed 'Gold Tokyo'. The experimental fish was starved for 24 hrs prior to the toxicity test. Standard methods (APHA, 1998) were used for conducting toxicity tests using white colored plastic tanks of 25 liter capacity. Probit analysis by Finney (1980) was carried out to calculate LC_{50} and it has been found out to be 17.236 mg/L for 96 hrs. The toxicity tests were conducted at different sub-lethal concentrations *viz*. 5.745 mg/ L ($1/3^{rd}$ of LC_{50}) and 2.462 mg/ L ($1/7^{th}$ of LC_{50}) prepared from the stock solution of arsenic trioxide manufactured by Qualikems Fine Chemicals Private Limited, New Delhi, India. Arsenic trioxide is not soluble in water as such; hence sodium hydroxide crystals were used to dissolve it. A batch of 15 fishes was exposed to above mentioned sub-lethal concentrations for a period of 30 days and 60 days respectively. A parallel control was maintained in toxicant free tap water. The temperature and pH were maintained during the experiment at 22 ± 2°C and 7 ± 0.4 respectively. Test water was replaced every 24 hrs to remove fish metabolites and to maintain the toxicant concentration. On 30^{th} and 60^{th} day of exposure period, fish were pithed and third gill arch from control and experimental fish was dissected out. The gill arches were fixed in 3% buffered

gluteraldehyde for 8 hrs at 4ºC, washed in 0.2 M phosphate buffer (pH 7.2), then dehydration was done in different grades of acetone, air dried and mounted on aluminum stubs with the help of double adhesive tape. The sample were sputter coated (100 A^0) with gold in sputter coater and then samples were observed under JEOL- 6100 SEM at 15-20 KV accelerating voltage. The SEM facility was provided at RSIC- CIL, Panjab University, Chandigarh as well as EM facility at AIIMS, New Delhi.

RESULTS & DISCUSSION

In the gills of fish in control group, large number of primary gill filaments are present on the gill arch (Fig. 1-a) with each row bearing large number of secondary lamellae (Fig. 1-b) showing typical leaf like structure. Each of secondary lamella shows the presence of large number of pavement cells (Fig. 1-c). The micrograph at higher magnification (Fig.1-c) shows the presence of numerous raised micro ridges with double ridged border of the pavement cells. These microridges increase the surface area for respiration. The present results on structure of normal gills fall exactly in line with earlier findings (Hossler *et al.,* 1979; Dunel-Erb and Laurent, 1980; Sawhney and Johal, 2000; Johal *et al*., 2007; Barillet *et al*., 2010; Liu *et al*., 2011).

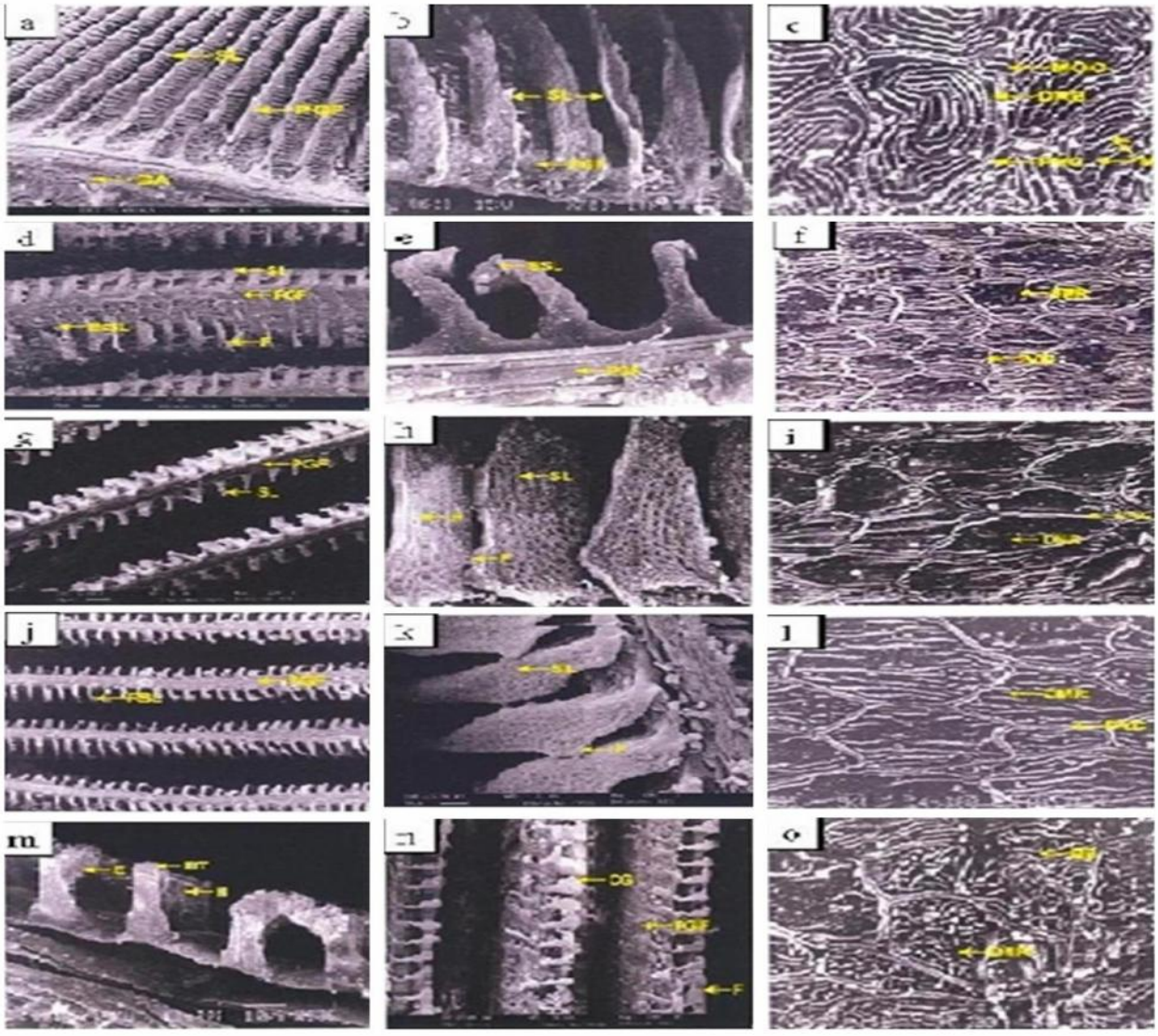

Fig. 1: (a-c) Scanning electron micrographs of gills of *Ctenopharyngodon idellus* (Val.) of control group. (d-f) photomicrographs of gills of *C. idellus* (Val.) treated with arsenic trioxide at a concentration of 5.745 mg/L (1/3rd of LC_{50}) for 30 days. (g-i) photomicrographs of gills of *C. idellus* (Val.) treated with arsenic trioxide at a concentration of 2.462 mg/L (1/7th of LC_{50}) for 30 days. (j-l) photomicrographs of gills of *C. idellus* (Val.) treated with arsenic trioxide at a concentration of 5.745 mg/L (1/3rd of LC_{50}) for 60 days. (m-o) photomicrographs of gills of *C. idellus* (Val.) treated with arsenic trioxide at a concentration of 2.462 mg/L (1/7th of LC_{50}) for 60 days.

Abbreviations: BSL: Bended secondary lamellae, **BT**: Broken tip of secondary lamellae, **BrSL**: Broken secondary lamellae, **Cr**: Cracks, **DMR**: Disrupted microridges, **DRB**: Double ridged border, **F**: Fusion, **GA**: Gill arch, **H**: Hyperplasia, **MB**: Marginal breakage **MGO**: Mucous gland opening, **PGF**: Primary gill filament, **PVC**: Pavement cell, **SL**: Secondary lamellae

On exposure of fish to concentration of 5.745 mg/L of arsenic trioxide, there appears curling as well bending of secondary lamellae (Fig. 1-d), fusion and complete sloughing off the structures from primary filament (Fig. 1-e). There also occurs a marked disruption of the microridges of pavement cells varying in degree (Fig. 1-f). On exposure for 30 days at 2.462 mg/L, there appears curling of secondary lamellae varying in degree in different primary gill filaments (Fig. 1-g), secondary lamella also showed hyperplasia resulting in swelling (Fig. 1-h) and also marked disruption of microridges (Fig. 1-i).

On prolonged exposure of gills to arsenic trioxide for 60 days at 5.745 mg/L, there occurs curling, bending and fusion of secondary lamellae (Fig. 1-j) along with hyperplasia of secondary lamellae (Fig. 1-k). Protrusion of microridges and swollen microridges are also seen in certain pavement cells and disruption in others (Fig. 1-l).

On exposure of gills at 2.462 mg/L concentration of arsenic trioxide for 60 days, certain secondary lamella showed broken globular tips (Fig. 1-m). At certain points, fusion of secondary lamellae while at other points, disruption of secondary lamellae was observed (Fig. 1-n). Microridges were also disrupted (Fig. 1-o).

In present work, bending as well curling of the secondary lamellae has been observed at low concentration (2.462 mg/L) at an exposure period of 30 days. Certain secondary lamellae showed hyperplasia at this concentration. On prolonging exposure period to 6o days, there appeared marked disruption of the secondary lamellae. Microridges also showed the damage at both the concentrations. At higher concentration of 5.745 mg/L, there appeared bending, curling in increasing magnitude as well as sloughing off the secondary lamellae from the primary gill filament. On prolonging exposure period upto 60 days, the degree of bending and curling became much more along with hyperplasia like condition in many of the secondary lamellae.

Gill surface alterations such as shrinkage, detachment of epithelium, degeneration of microridges had also been reported due to various stress conditions (Fanta *et al.*, 1995; Sawhney and Johal, 2000; Machado, 2003; Athikesavan *et al.*, 2006; Chezhian *et al*., 2010). Hyperplasia had been reported in epithelial cells, which is a defensive mechanism as it reduces toxic agent penetration by increasing distance between blood and polluted water (Morgan and Tovell, 1973; Mallat, 1985; Wong *et al*., 2012). According to Johal and Dua (1994), hyperplasia increases respiratory diffusion distance causing stress and asphyxiation in fish. Altered structure of microridges created problem in delivery of oxygen to gill epithelium. Loss of typical microridges pattern had also been reported in various fishes by different workers (Sawhney and Johal, 2000; Ba-Omar *et al*., 2011).

Therefore, the present study revealed that the gills of fish are quite susceptible to high degrees of damage even at low concentration of pesticides/ insecticides, so pesticides/ insecticides should be cautiously used.

ACKNOWLEDGEMENTS

Authors are thankful to Chairperson, Department of Zoology, Panjab University, Chandigarh for providing facilities required during the period of experiment. We are also thankful to RSIC- CIL, Panjab University, Chandigarh as well as EM facility at AIIMS, New Delhi.

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