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Branchial Histopathological Study of Catfish *Heteropneustes fossilis* Following Exposure to Purified Neem Extract, Azadirachtin

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Abstract: This study was carried out to investigate the effects of purified neem extract azadirachtin on the gill histology of *Heteropneustes fossilis* for short- and long-term. The common alterations observed were - over-secretion of mucous, hyperplasia and fusion of gill lamellae, epithelial uplifting and necrosis of gill epithelial cells. These changes were dose and time-dependent. It was concluded that although botanical pesticides are being considered as less toxic/safe, but it may provoke morphological changes in vital organs of the fish such as gill. Hence, precautions must be taken into account when botanicals are being used in fish inhabiting areas.

Key words: Neem · Azadirachtin · Gill · Fish

INTRODUCTION

The aquatic environment is currently under threat by the indiscriminate use of synthetic pesticides by the human activities and causing high risk to non-target organisms. One of the most important non-target inhabitants of the aquatic ecosystem affected by pollution are fish - both freshwater and marine. Fish are exposed to aquatic toxicants through their extensive and delicate respiratory surface of the gills and as in case of seawater fish, also via drinking. Gills participate in very important functions such as respiration, osmoregulation and excretion and are also the contaminant depuration site, whereas the detoxification and metabolism of toxic agents occurs. Natural pesticidal products, also called botanical pesticides, are available as alternatives to synthetic chemical formulations. One of the most promising botanical pesticide is azadirachtin (AZA) extracted from the neem tree (*Azadirachta indica* A. Juss.). This compound has been used for the last 2000 years as antiviral, antibacterial and antifungal compound [1, 2]. Neem extract has also been reported for the control of pests [3] and in aquaculture practices for the control of fish predators [4].

Neem extract has been considered as possessing low toxicity to the non-target aquatic life [5]. Omoregic and Okpanachi [6] have reported that aqueous extracts of the bark of neem plant provoked respiratory problems in *Tilapia zilli*. To the best of our knowledge there is no existing report on the histological alterations in the gills of fish after exposure to purified azadirachtin. Hence, in this study we have investigated such study on the gills of a freshwater teleost *Heteropneustes fossilis* after treatment with various doses of purified azadirachtin (Ozoneem Aza).

MATERIALS AND METHODS

Live specimens of adult freshwater catfish, *Heteropneustes fossilis* (both sexes, body weight 34-52 g) were collected locally and acclimatized to the laboratory conditions for 15 days in plastic tanks. The physicochemical characteristics of the tap water used in the experiment were- temperature 24.70 ± 1.65°C. pH 7.25 ± 0.09; hardness 167 ± 5.72 mg L^{-1} as $CaCO_3$; electrical conductivity 306 ± 69 μmho cm^{-1}; dissolved oxygen 7.84 ± 0.38 mg L^{-1} and no free chlorine. During acclimatization the fish were fed daily with wheat flour pellets and ground dried shrimps, 2-3 times per day. Water was renewed daily. The fish were not fed 24 h before and during the experimental period. The study was approved by the Animal Research Ethical Committee of DDU Gorakhpur University.

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In the present study, purified neem extract Ozoneem Aza (containing azadirachtin A 23.78% and azadirachtin B 3.59%; Batch No. AZA-351, manufactured by Ozone Biotech, India) was used. In short-term exposure the fish were subjected to 0.8 of 96 h LC_{50} value of azadirachtin (41.89 mg L^{-1}) for 96 h. In long-term exposure the experiment was performed for 28 days by using 0.2 of 96 h LC_{50} value of azadirachtin (10.47 mg L^{-1}). Concurrently, a control group was also used for comparison by using the tap water containing alcohol. Fish were kept in groups of 10 in 40-L media. Azadirachtin was firstly dissolved in alcohol and then added to tap water to obtain the desired concentration. Six fish were killed on each time intervals from control and experimental (azadirachtin) groups after 24, 48, 72 and 96 h in short-term exposure and after 7, 14, 21 and 28 days in long-term experiment.

Gills were fixed in aqueous Bouin's solution on the above mentioned exposure periods and processed routinely for histological studies. Sections were cut at six μm and stained with hematoxylin-eosin (HE) for light microscopic examination (Olympus CH 20i). Photomicrographs were taken with the aid of Olympus E 420 camera.

RESULTS

In control fish, single layer of epithelial cells supported by pillar cells has been noticed in gill lamella. A thin layer of basement membrane separates the epithelial cells and pillar cells (Fig. 1). Mucous cells are intercalated with epithelial cells.

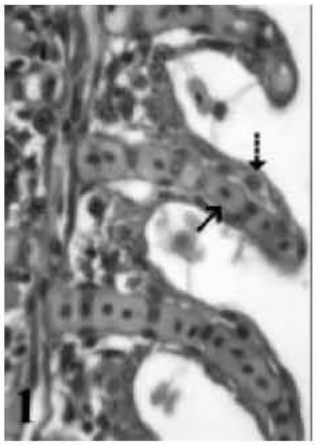

Fig. 1: Gill of control *Heteropneustes fossilis* showing pillar cells (arrow) and epithelial cells (broken arrow). HE x 500

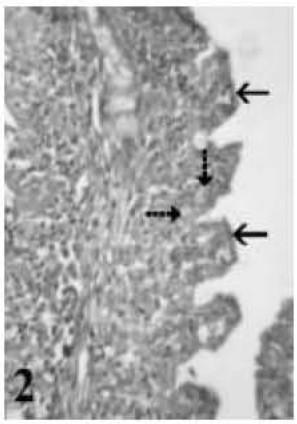

Fig. 2: Gill of 48 h azadirachtin (41.89 mg L^{-1}) treated fish showing swelling at tip of secondary lamellae (arrows) and activated mucous cells on lamellar apex (broken arrows). HE x 200

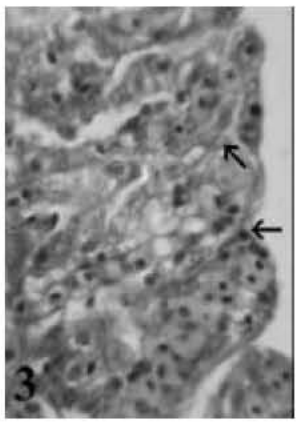

Fig. 3: Fusion of secondary gill lamellae (arrows) in 72 h azadirachtin (41.89 mg L^{-1}) treated fish. HE x 500

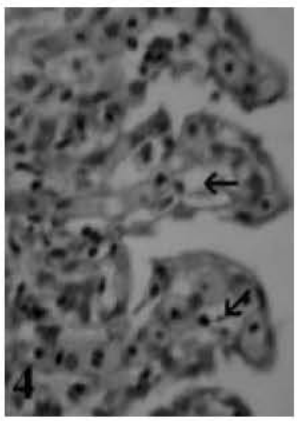

Fig. 4: Detachment of secondary lamellar epithelium (arrows) from the pillar cells after 72 h azadirachtin (41.89 mg L^{-1}) treatment. HE x 500

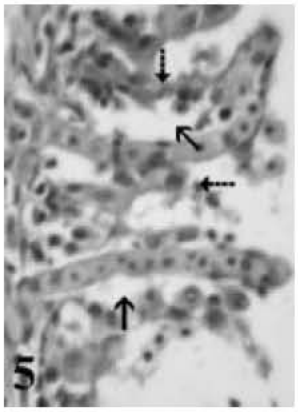

Fig. 5: Necrotic cells (broken arrows) and separation of secondary gill lamellae from pillar cells (arrows) after 96 h azadirachtin (41.89 mg L^{-1}) treatment. HE x 500

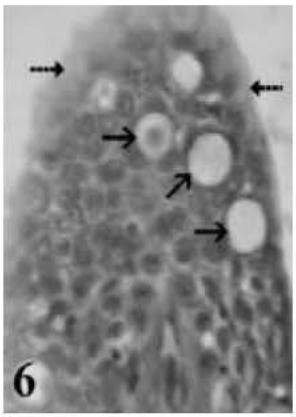

Fig. 6: Gill of 7 day azadirachtin (10.47 mg L^{-1}) treated fish showing thin coating of mucous (broken arrows) on gill lamellae and activated mucous cells (arrows). HE x 800

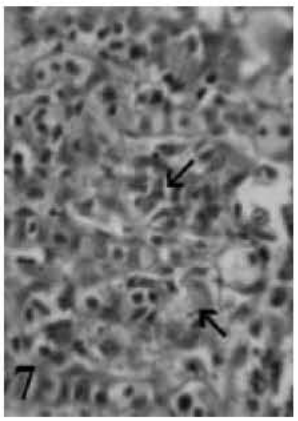

Fig. 7: Fusion of lamellae (arrows) after 14 day azadirachtin (10.47 mg L^{-1}) treatment. HE x 500

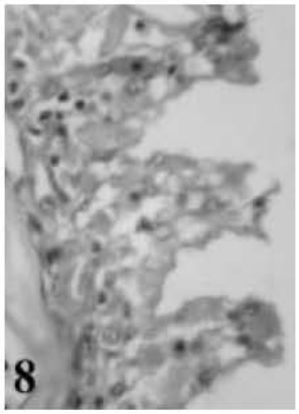

Fig. 8: Ragged appearance of gill lamellae after 14 day azadirachtin (10.47 mg L^{-1}) treatment. HE x 500

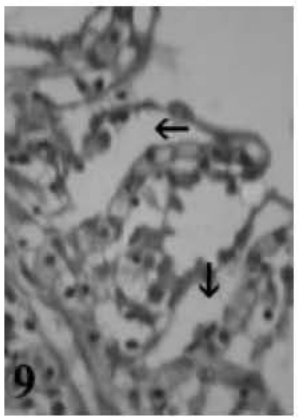

Fig. 9: Gill of 21 day azadirachtin (10.47 mg L^{-1}) treated fish showing separation of epithelial cells from pillar cells (arrows). Note degenerating cells also. HE x 500

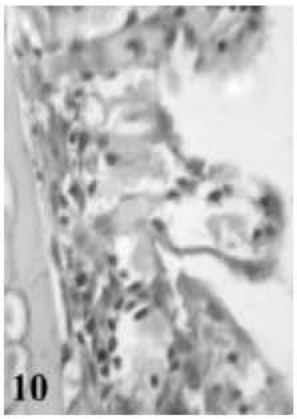

Fig. 10: Completely degenerated gill lamellae and necrotic cells after 28 day azadirachtin (10.47 mg L^{-1}) treatment. HE x 500

The mucous cells become active after 24 h following azadirachtin treatment. After 48 h azadirachtin treatment, swelling at the tips of several secondary lamellae has been observed (Fig. 2). The mucous cells have been noticed to discharge their content and they get activated on the lamellar apex (Fig. 2). Fusion of secondary gill lamellae occurs after 72 h azadirachtin treatment (Fig. 3). Detachment of secondary lamellar epithelium from the pillar cells has been recorded at certain places (Fig. 4). After 96 h treatment, necrotic cells are encountered and increased frequency of separation of secondary gill lamellae from pillar cells has been observed (Fig. 5).

After 7 day azadirachtin treatment, a thin coating of mucous is seen on the gill lamellae. The mucous cells are seen more activated (Fig. 6). Following 14 day azadirachtin treatment, there is hyperplasia of the gill lamellae resulting into fusion of the lamellae (Fig. 7) and at certain places, ragged appearance of gill lamellae are noticed (Fig. 8). After 21 day azadirachtin treatment, there is sloughing of the respiratory epithelium and separation of epithelial cells from pillar cells (Fig. 9). Several degenerating cells are also encountered (Fig. 9). After 28 day azadirachtin treatment, there occur more necrotic cells and at places gill lamellae are completely degenerated (Fig. 10).

DISCUSSION

In the present study, gill lesions were observed in *H. fossilis* after azadirachtin treatment. However, the degrees of alterations were positively related with the concentration of azadirachtin and the duration of exposure. Common gill lesions observed in azadirachtin treated *H. fossilis* were hypertrophy of mucous cells, hypertrophy of the respiratory epithelium, fusion of the adjacent gill lamellae due to hyperplasia of respiratory epithelium, separation of the epithelium from lamellar supporting cells, necrosis and degeneration of gill epithelium.

In the gill of azadirachtin treated fish, hypertrophy of mucous cells has been observed. Similar activity of the mucous cells has been reported earlier by treatment with other pesticides to the fish- carbamate [7]; Endrin [8] and chlorpyrifos [9]. The over-secretion of the mucous cells and forming a mucous layer at few places covering the gill lamellae can be considered as a defensive mechanism to combat the effects of the toxicants.

In azadirachtin exposed *H. fossilis* hyperplasia, fusion of gill lamellae and separation of epithelial cells (epithelial lifting) have been observed. This is in conformity with other investigators who have also observed similar alterations in the gills of the fish exposed to aquatic contaminants [9-21]. Gill hyperplasia has been suggested as a defensive mechanism resulting into a decrease in the respiratory surface and increase the distance between external environment and the blood, thus forming a barrier to the entrance of contaminants [16, 22]. Camargo and Martinez [17] have opined that as a consequence of the increased distance between water and blood caused by epithelial lifting the oxygen uptake is impaired.

The necrosis observed in the gills of azadirachtin treated fish supported the findings of other investigators who have also observed similar degeneration after treatment with toxicants [11, 12, 13, 16]. The necrosis of gill epithelium seems to be a direct response to azadirachtin.

It was concluded that although botanical pesticides are being considered as less toxic/safe, but it may provoke morphological changes in vital organs of the fish such as gill. Hence, precautions must be taken into account when botanicals are being used in fish inhabiting areas.

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