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Research Article

HISTOPATHOLOGICAL ALTERATIONS IN THE INTESTINE OF FISH *CHANNA PUNCTATUS* EXPOSED TO SUBLETHAL CONCENTRATIONS OF ATRAZINE

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ABSTRACT

Atrazine commonly used as a herbicide, contaminates the aquatic ecosystem as a toxic pollutant from agricultural and domestic washouts. The aim of the present study was to investigate the toxic effect of Atrazine on intestine tissues in the fish *Channa punctatus* exposed to sublethal concentration of 5 mg/L, 10 mg/L and 15 mg/L for 15 and 30 days respectively. The histological observations of the intestine showed deleterious effect of Atrazine exposure time, the intestine of *C. punctatus* showed many strong changes viz, rupturing of longitudinal and circular muscle layers, reduction of villi, Necrosis at the tips of villi and highly shrinkage of sub mucosa at the tips of the villi were observed, Distortion of goblet cells, Blood vessels and Loss of structural integrity of mucosal folds were also seen in the intestine of *C. punctatus* at higher exposure of Atrazine. This clearly demonstrates that the sublethal concentrations of Atrazine have a deleterious effect on the intestine of *C. punctatus*. So, the herbicide Atrazine can be considered as a potent toxic pollutant capable of destroying the balance of the aquatic ecosystem.

Keywords: *Channa Punctatus*, Atrazine, Intestine, Pollutant, Herbicide.

INTRODUCTION

Large-scale application of pesticides to crops and plants may contribute to the presence of toxic substances in the environment. These toxicants through winds, floods, rains and surface run off reach unrestricted areas like ponds, springs and rivers thus producing an adverse impact on the aquatic biota including fishes (Rahmathullah *et al*., 2003). Atrazine (2-chloro-4-ethylamino-6-isopropylamino-1, 3, 5-triazine) is one of the most widely used triazine herbicides in the world. It is used to control broad- leaf weeds in corn or crops, including green vegetables, weeds in maize, sorghum, asparagus, citrus, sugar cane, bananas, coffee, oil palms and grass land/forestry (Cui *et al*., 2002).The major uses being maize and sorghum. It is also used in combination with many other herbicides (Alvarez & Fuiman, 2005; Roberts *et al*., 1998; Steinberg, 1997; Zhou *et al*., 2008).

After spraying on crops, it can enter watercourses, because of its high mobility through soil (Waring & Moore, 2004). Hussain *et al*. (1996) pointed out that Atrazine reaches aquatic environments due to proximities of the agricultural country sides to the water places, or directly due to the careless application in such environments. After reaching the environment and ultimately affect non-target organisms such as fish (Bhatnagar & Srivastava, 1992).

In the present study, the freshwater teleost fish, *Channa punctatus* locally called karra is an important and popular fish species in the water bodies located in Jabalpur regions of Madhya Pradesh (India) is selected. This region has been traditionally well developed agricultural area mostly cultivating irrigated wet crops. In view of the incidence of herbs and pests, a variety of herbicides and other chemicals are used on crops for controlling the agriculture pests. The surface runoff from these areas during monsoon flow into the ponds and affect the aquatic fauna mostly fish.

The purpose of present study was to access the Histopathological changes in the intestine of *C. punctatus*

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exposed to atrazine which is commonly used to control the herbs of several agricultural fields in India.

MATERIAL AND METHODS

Live specimens of test organism *C. punctatus* were purchased from Hanumantal fish market (Jabalpur) M.P. Average length and weight of the experimental fish was 10 to 14 cm in length and 50 to 80 gms in weight. The experiments were conducted in the laboratory using glass aquarium (40 × 27 × 26 cm). The fishes were dipped in tubs and were disinfected with 0.01% of $KMnO_4$ solution and washed thoroughly to prevent dermal infection and the fishes were maintained in aquariums with dechlorinated water which was continuously aerated. These fishes were acclimatized in the laboratory for two weeks prior to the experimentation.

For sub-acute toxicity, the various sub lethal concentrations of Atrazine viz. 5mg/L, 10 mg/L and 15 mg/L for 15 and 30 days respectively were given. Sets of twelve fishes were transferred to four different aquaria. Out of four, one served as control (contained only dechlorinated water) and rest three contained different concentrations of Atrazine. Six fishes were taken out from each aquarium after Fifteen days and the rest of the fishes were taken out after 30 days.

The fish were sacrificed and intestine was removed for histological examination. The tissues were washed in saline water and preserved in plastic vials with Bouin's fluid for at least 18 hours. The intestine was then dehydrated, embedded in paraffin wax. The embedded blocks were sectioned (6 µ) by a microtome machine (Buffalo. N. Y. 14215. U. S. A.) Fixed and prepared slides were kept for one night. Then the sections were stained with haematoxylin and eosin and were then mounted for later examination. Changes induced by treatment in the intestinal tissues were photographed and analyzed by light microscope at 10 X eye piece magnification and 40X objective magnification (Olympus model U-CMAD3) with Camera attachment of Samsung (model SDC-313B).

RESULT AND DISCUSSION

The innermost layer is mucosa consists of (CE) columnar epithelium with columnar cells, (GC) goblet cells, and (LP) Lamina propria, Inner to the muscle layer is (SM) sub mucosa, which consists of connective tissue, blood vessels and nerve. It permits mobility of the mucosa. Muscular is below the (S) serosa and it consisting of inner layer of circularly oriented and outer layer of longitudinally oriented smooth muscle, circular muscle layer (CML) and longitudinal layer (LML). The functions are mixing of food with digestive enzymes.

Adventitia is relatively dense layer of areolar connective tissue often blending with connective tissue of surrounding structures ,when covered by the peritoneum it is also called a (S) Serosa, containing blood vessels, nerves, and lymphatics also consists of flattened cell covering the outer boundary. The histological sections of Figure 1 the control intestine of *C. punctatus* consists of four layers: Mucosa, sub mucosa, muscularis, adventia. Figure 2. At lower concentrations 5 mg/L for 15 days not many changes were observed, Section of intestine showing loosely arranged of circular muscle layer and necrosis of epithelial cells were also observed. No changes in villi and longitudinal muscular layer were noticed (Figure 3 and 4). However higher concentration of Atrazine 10 mg/L and 15 mg/L for 15 days showed many changes rupturing of muscular layers both and circular muscle layer, Necrosis were prominent, reduction of villi, Reductions in the length of intestinal fold were obvious Figure 5. Slight changes in Intestine were observed after exposure to 5 mg/L for 30 days, degeneration of serosa, vacuolization in certain places and loosely arrangement of longitudinal muscle were also observed, Figure 6 and 7. After exposure to 10 mg/L and 15 mg/L for 30 days, the intestine of *C. punctatus* showed many strong changes viz, rupturing of longitudinal and circular muscle layers, reduction of villi, Necrosis at the tips of villi and highly shrinkage of sub mucosa at the tips of the villi were observed, Distortion of goblet cells, Blood vessels and Loss of structural integrity of mucosal folds were also seen in the intestine of *C. punctatus* at higher exposure of Atrazine.

Intestine is the most important part of the fish alimentary canal and plays an important role in digestion and absorption of food materials as well as considered as a sensitive organ for toxicity assessment of xenobiotic substances in fish species as they are directly exposed to complex mixture of toxic substances via ingestion of contaminated food stuffs or indirectly via blood and/or lymph (Muniyan, 1999). In the present study histopathology of the intestine of *C. punctatus* was studied after Atrazine intoxication. A number of studies on histopathological effects of different pesticides on fish intestine have been reported by Velmurugan *et al*. (2007) who reported degeneration in the tip of villi, loss of structural integrity in mucosal folds, hypertrophy, vacuolation and necrosis in *Cyprinus carpio* and *Cirrhinus mrigala* after exposed to atrazine. Sharma *et al*., (2001) also reported similar results in fish *C. Batrachus* and *C. mrigala* after pesticidal exposure. Ravanaiah & Murthy, (2010) also noticed vacuolations, lesions in villi and serosa layer, necrosis, congestion in blood capillaries and severe mucus secretion in *Tilapia mossambica* when exposed to industrial pollutants. Yildirim *et al.* (2006) also reported similar result in fish *Oreochromis niloticus* after exposed to pesticide. Reports on degenerative changes and rupture in tip of villi, loss of structural integrity of mucosal folds and degeneration and necrosis of submucosa in the intestine of *C. punctatus* after the exposure to carbofuran was also found by Muley *et al.* (1996).

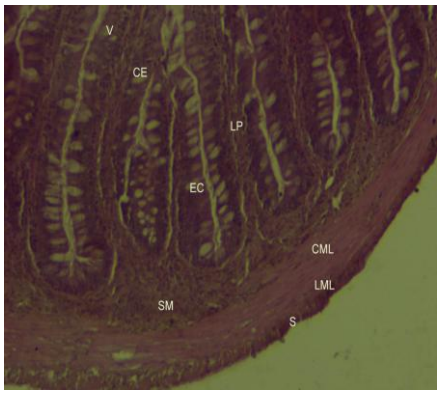

Figure 1

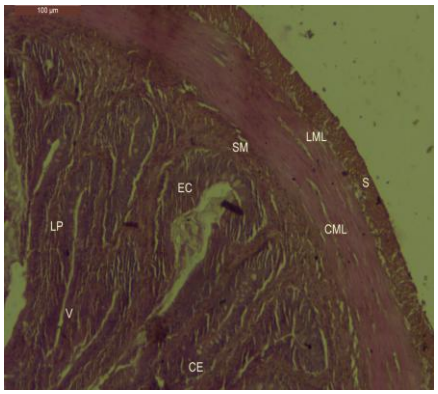

Figure 2

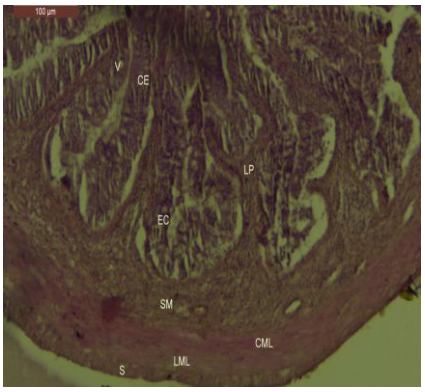

Figure 3

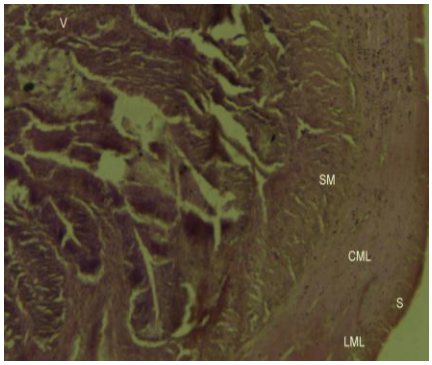

Figure 4

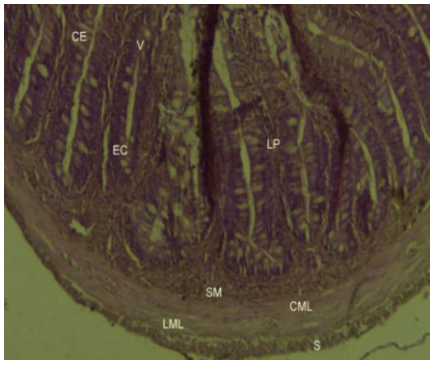

Figure 5

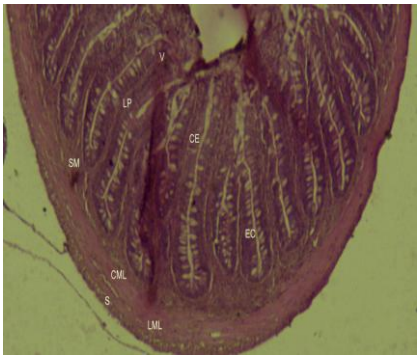

Figure 6

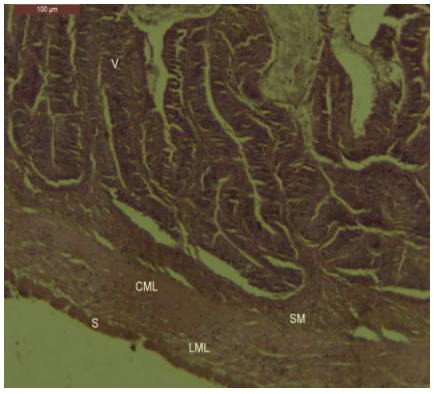

Figure 7

Figure 1. Transverse section of the intestine of the control fish *Channa punctatus* showing the normal organization of intestine. Columnar Epithelium (CE), sub mucosa (SM) and serosa (S) Bouin, 6 µm, HE. X 400.

Figure 2. Intestine of *C. punctatus* exposed for 15 days to sublethal concentration (SLC) 5 mg/L of Atrazine; Bouin, 6 µm, HE x400.

Figure 3. Intestine of *C. punctatus* exposed for 15 days to sublethal concentration 10 mg/L of Atrazine; Bouin, 6 µm, HE x400.

Figure 4. Intestine of *C. punctatus* exposed for 15 days to sublethal concentration 15 mg/L of Atrazine ; Bouin, 6 µm, HE x400.

Figure 5. Intestine of *C. punctatus* exposed for 30 days to sublethal concentration 5 mg/L of Atrazine; Bouin, 6 µm, HE x400.

Figure 6. Intestine of *C. punctatus* exposed for 30 days to sublethal concentration 10 mg/L of Atrazine; Bouin, 6 µm, HE x400.

Figure 7. Intestine of *C. punctatus* exposed for 30 days to sublethal concentration 15 mg/L of Atrazine; Bouin, 6 µm, HE x400.

CONCLUSION

The results obtained by the study on toxic effect of Atrazine clearly demonstrate that, at sublethal concentrations itself the herbicide has deleterious effect on the tissues exposed to it directly or even indirectly. Hence, Atrazine can be considered as a potent toxic pollutant capable of destroying the balance of aquatic ecosystem.

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