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Intestinal Histopathology as a Biomarker for Lindane Toxicity in Teleost Fish, *Channa punctata*

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Abstract

Our study aims to assess the pathological alterations in the intestinal issues of freshwater teleost, *Channa punctata*, at weekly exposure durations, for 21 days. A sublethal dose of 0.03 g L^{-1} was selected and test and control fish were maintained for 21 days under laboratory condition. Light microscopic study, with Haematoxylin and Eosin stain, was used to detect the intestinal pathology. The study revealed structural damages and a direct correlation between pesticide exposure and severity of effects.

***Keywords**: pesticide; toxicity; biomarker; histopathology*

1. Introduction

Lindane is a persistent organochlorine pesticide that had widespread usage in India until a total restriction was made in 2014 (www.cibrc.nic.in). However, the existing stock pile is still reported in use in many parts of the country against agricultural and farm animal pest, wood preservatives, as well as against human lice and scabies treatment. Lindane, like many pesticides, can reach natural waters either via transfer of the chemicals from soil or directly by spraying against target organisms (Oruc, 2010). Lindane is hydrophobic and and its isomers readily volatilize and bio accumulate in fatty tissues of animals (Willett *et al.,* 1998). Several studies reported the presence of lindane residues in river, soil and groundwater in India (Sarkar *et al.*, 2003; Singh, 2001). Lindane is also highly toxic to aquatic organisms (EXTOXNET, 1996). In fact, fish as the non target victims of lindane have been reported in several studies (Oliva *et al.,* 2008; Saravanan *et al.,* 2011). Evaluation of ecotoxicological risks caused by pesticides is based on the toxic effects to non-target organisms like fish (Golow and Godzi, 1994). Fish are used as excellent indicator of aquatic pollution due to their high sensitivity to environmental contaminants which may damage certain physiological and biochemical processes when contact with the organs of fishes (Mahttiessen *et al.,* 1995). Indeed, histopathological biomarkers provide a rapid method to detect effects of pesticides (Johnson *et al.,* 1993). The exposure of fish to chemical contaminants is likely to induce a number of lesions in different organs (Bucke *et al.,* 1996) includine intestine (Das and Gupta, 2013 a, b). The intestine is a complex multifunctional organ. In addition to digesting and absorbing feedstuffs, the intestine is critical for water and electrolyte balance, endocrine regulation of digestion and metabolism, and immunity. Hence, the present study aims at estimating the impacts of sublethal dose of lindane on the intestine of *Channa punctata*, at weekly intervals.

2. Material and Methods

2.1 Pesticide

Stock solution of commercially available lindane was prepared from 6.5% ɤ BHC (Trade name -Kunahex) procured from Kundu Agro Chemicals Ltd., Kolkata, India, and purchased from a local pesticide shop. The stock solution was prepared by dissolving 50 g of lindane in 1 litre of tap water.

2.2 Fish specimen

Healthy *Channa punctata* (length, 16.58 ± 0.3cm, and weight, 25.7 ± 0.5 g) were procured from a local fishery near Assam University campus with no reported pollution load. They were acclimatized for 3 weeks in a large cement tank (1000 L capacity) under laboratory conditions. During the acclimatization period, fish were fed ad libitum with minced goat liver twice daily.

2.3 Design of experiment

From the stock solution, sublethal concentration of 0.03 g/L lindane (96 h LC_{50} was reported elsewhere to be 2.25 g/L) was prepared. 15 fish were taken out of the acclimatization tank and placed in a 75 L glass aquaria containing 0.03 g/L lindane. The test media was replaced every alternate day. 5 fish each were dissected at 7, 14 and 21 days intervals; the intestinal tissues were fixed in 10% formaldehyde and embedded in paraffin for microscopic examination. Various sections (5µ) were obtained, stained with haematoxylin and eosin and examined under a light microscope. A search was carefully conducted to check for the presence of alterations. The experiments were performed as per the guidelines recommended by Assam University Ethical Committee for treatments of experimental animals.

3. Results and Discussion

The intestine of *C punctata* shows four layers of tissues namely serosa, muscularis, submucosa and mucosa. The outermost serosa is followed by a well developed muscularis (longitudinal and circular muscle) embedded in loose connective tissue richly supplied with blood capillaries (Fig. 1C). The control intestine showed normal architecture

throughout the study period. However, 0.03 g L^{-1} lindane exposed tissues were subjected to stress that was reflected in various histopathological changes. Intestine showed a specific duration specific responses which included inflammatory cell infiltration, vacuolation of epithelial cell, proliferation of goblet cells and after 21 days of exposure, necrotic areas were evident. (Fig 1, 7d, 14d, 21d).

The intestine is the first organ the come into contact with food-borne contaminants (Braunbeck and Appelbaum 1999). Braunbeck and Appelbaum (1999) also found that in the intestine, exposure to endosulfan is associated with changes in the epithelial lining, which indicated disturbance of intestinal absorption. Cengiz *et al.* (2001) reported oedema, degeneration, accumulation of lymphocytes in the lamina propria, pycnotic state of nuclei, and necrosis in the intestine of *Gambusia affinis* exposed to endosulfan. The intestine of *Cyprinus carpio* exposed to chlorinated pesticides aldrin, dieldrin, BHC and DDT showed fusion of intestinal folds and acute epithelial necrosis (Satyanarayan *et al.*, 2012). Das and Gupta (2013a) found superficial erosion of mucosa, dense lamina propria, chronic inflammatory cell infiltration, vacuolation and haemorrhage of mucosa and submucosa in *Esomus danricus* exposed to sublethal concentrations of endosulfan. Mandal and Kulshrestha (1980) found lesion formation in villi of *Clarias batrachus* after exposure to sumithion. Necrosis, infiltration of lymphocytes and eosinophils were reported in the intestine of *Gambusia affinis* exposed to deltamethrin (Cengiz and Unlu, 2006).

The present study, thus, concludes that lindane, even at a very low dose of 1/100th of 96-h LC_{50} value of a commercial formulation, which is only 6.5% of the active ingredient, has the potential to induce histopathological lesions in the intestine. Hence, there is an urgent need to completely phase out the domestic stockpile of lindane, to ensure minimum ecotoxicological damage of aquatic fauna.

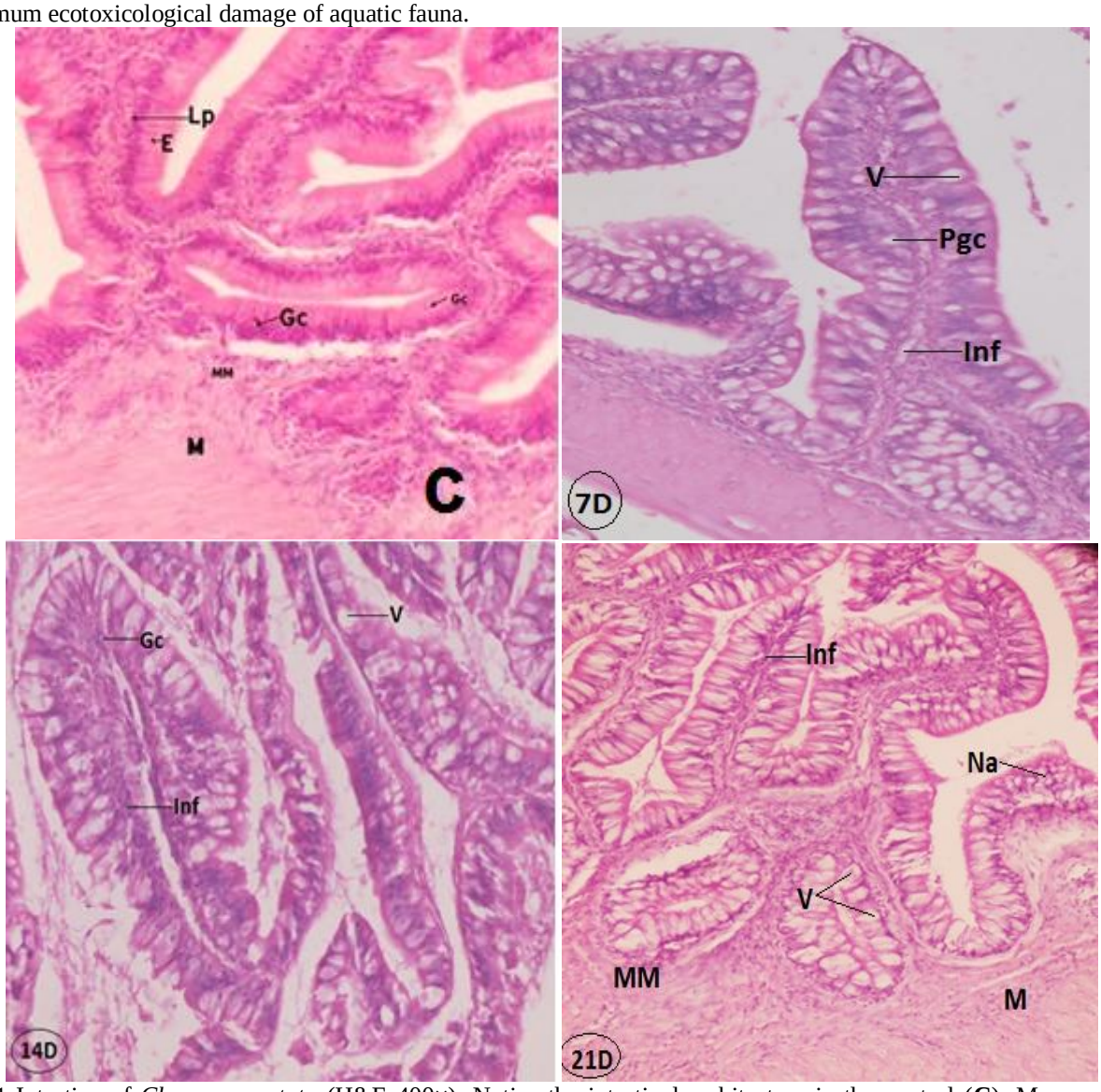

Fig. 1 Intestine of *Channa punctata* (H&E 400×), Notice the intestinal architecture in the control (**C**); M-muscularis, MM-muscularis mucosa, Lp-lamina propria, E-epithelium, Gc-goblet cell (**7d, 14d, 21d**) Intestine of fish exposed to 0.03 g L^{-1} lindane (H&E 400×); for 7, 14 and 21d respectively; V- vacuolation of epithelial cell, Pgc-proliferation of goblet cells, na-necrotic areas. inf-inflammatory cell infiltration.

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References

Braunbeck, T. and Appelbaum, S. (1999). Ultrastructural alterations in the liver and intestine of carp *Cyprinus carpio* induced orally by ultra-low doses of endosulfan. Dis. Aquat. Organ. 36: 183–200.

Bucke, D. Vethaak, D. Lang, T. and Mellergaard, S. (1996). Common diseases and parasites of fish in the North Atlantic: training guide for identification, International Council for the Exploration of the Sea Techniques in Marine Environmental Sciences , Copenhagen.

Cengiz, E. Unlu, E. and Bale, K. (2001). The histopathological effects of thiodan on the liver and gut of mosquito fish, *Gambusia affinis*. J. Environ. Sci. Health B. 36: 75-85.

Cengiz, E.I. and Unlu, E. (2006). Sublethal effects of commercial deltamethrin on the structure of the gill, liver and gut tissues of mosquito fish, *Gambusia affinis*: a microscopic study. Envtl. Toxicol. Pharmacol. 21: 246–253.

Central Insecticide Board and Registration Committee. Insecticides banned, restricted. www.cibrc.nic.in downloaded on 01.09.2014

Das, S. Gupta, A. (2013a). Histopathological changes in the intestine of Indian flying barb (*Esomus danricus*) exposed to organochlorine pesticide, endosulfan (EC 35). Int. J. Sci. Res. 2: 206-208.

Das, S. and Gupta, A. (2013b). Histopathological changes in the intestine of Indian flying barb (*Esomus danricus*) exposed to organophosphate pesticide, malathion (EC 50). Global J. Biol. Agri. Health Sci. 2: 90-93.

EXTOXNET (1996). Extension Toxicology Network, A pesticide information project of cooperative extension offices. USDA/Extension Service/National Agricultural Pesticide Impact Assessment Program.

Golow, A.A. and Godzi, T.A. (1994). Acute toxicity of deltamethrin and dieldrin to *Oreochromis niloticus* (Lin). Bull. Environ. Contam. Toxicol. 3: 351–354.

Johnson, L.L. Stehr, C.M. Olson, O.P. Myers, M.S. Pierce, S.M. Wigren, C.A. McCain, B.B. and Varanasi, U. (1993). Chemical contaminants and hepatic lesions in winter flounder (*Pleuronectes americanus*) from the northeast coast of the United States. Environ. Sci. Technol. 27: 2759–2771.

Mahttiessen. P. Sheahan, D. Harrison, R. Kirby, M. Rycroft, R. Turnbull, A. Volkner, C. and William, R. (1995). Use of a *Gammarus pulex* bioassay to measure the effects of transient carbofuran run-off from farmland. Ecotoxicol. Environ. Saf. 30: 111–119.

Mandal, PK. and Kulshrestha, A.K. (1980). Histopathological changes induced by the sub lethal sumithion in *Clarias batrachus* (Linn.). Indian J. Exp. Biol. 18: 547–552.

Oliva, M. Garrido, C. Sales, D. and de Canales, M.L.G. (2008). Lindane toxicity on early life stages of gilthead seabream (*Sparus aurata*) with a note on its histopathological manifestations. Envtl. Toxicol. Pharmacol. 25: 94–102.

Oruç, E.Ö. (2010). Oxidative stress, steroid hormone concentrations and acetylcholinesterase activity in *Oreochromis niloticus* exposed to chlorpyrifos. Pestic. Biochem. Physiol. 96: 160–166.

Sarkar, U.K. Basheer, V.S. Singh, A.K. and Srivastava, S.M. (2003). Organochlorine pesticide residues in water and fish samples: first report from rivers and streams of Kumaon Himalayan region, India. Bull. Environ. Contam. Toxicol. 70: 485–493.

Saravanan, M. Prabhu Kumar, K. and Ramesh, M. (2011). Haematological and biochemical responses of freshwater teleost fish *Cyprinus carpio* (Actinopterygii: Cypriniformes) during acute and chronic sublethal exposure to lindane. Pestic. Biochem. Physiol. 100: 206–211.

Satyanarayan, S. Satyanarayan, J.P.K.A. and Verma, S. (2012). Histopathological changes due to some chlorinated hydrocarbon pesticides in the tissues to *Cyprinus carpio*. IOSR J. Pharmacy. 2: 60-66.

Singh, R.P. (2001). Comparison of organochlorine pesticide levels in soil and ground water of Agra, India. Bull. Environ. Contam. Toxicol. 67: 126–132.

Willett, K.L. Ulrich, E.M. and Hites, R.A. (1998). Differential toxicity and environmental fates of hexachlorocyclohexane isomers. Environ. Sci. Technol. 32: 2197-2207.