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HISTOPATHOLOGICAL CHANGES IN THE INTESTINE OF INDIAN FLYING BARB (*ESOMUS DANRICUS)* EXPOSED TO ORGANOPHOSPHATE PESTICIDE, MALATHION (EC 50)

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Abstract

Indian flying barb (*Esomus danricus*) was exposed to three sublethal concentrations of malathion (EC 50) for 28 days and intestinal histopathology was observed by light microscopy after staining with Haematoxylin-Eosine. In the exposed fishes, chronic inflammatory cell infiltration (lymphocyte) along with ulceration of mucosa and vacuolation was observed. Higher doses had severe effects.

Keywords: *intestine, inflammation, vacuolation, teleost, organophosphate.*

1. Introduction

Malathion (diethyl [(dimethoxy phosphino thioyl] butanediotae) is an organophosphate pesticide commonly applied for public health (De Guise *et al.*, 2004) and control of agricultural pests (EPA, 2000). Malathion is highly toxic to fish (Mohan, 2000) due to the lack of hydrolytic enzymes in fish (Arecchon and Plumb, 1990). Malathion appears to inhibit AChE activity (Savolainen, 2001). In North Eastern India, Indian flying barb, *Esomus danricus* (Hamilton-Buchanan), a teleost fish, mostly inhabits water bodies near agricultural fields. Malathion, as well as other pesticides, used in agriculture find their way with runoff water interfering with all the metabolic processes and get accumulated in vital organs thereby affecting the functional activity of both exocrine and endocrine systems of non-target aquatic organisms including fish (Sahai, 1987). Malathion is also commonly found at low concentrations in rivers, streams and drinking water (Hoffman *et al.*, 2000; Larson *et al.*, 1999), thus affecting fish as well as mammals. The gastrointestinal system of fish is very much vulnerable to ingested toxic substances. As very little work has been done on effects of malathion on fish intestine, the present study assesses the histopathological effects of this pesticide on Indian flying barb intestine.

2. Materials and Methods

Fishes of similar length (46.77 ± 4.30 mm) and weight (0.86 ± 0.16 g) were collected from unpolluted, freshwater ponds near Assam University campus, Barak valley, South Assam, India (Das and Gupta, 2009). They were acclimatized under laboratory conditions seven days prior to experimentation. Temperature, pH, hardness and dissolved oxygen under laboratory condition were 29°C, 6.8, 30 mg l^{-1} and 5.5 mg l^{-1} respectively. Stock solution of commercial grade Devimalt (malathion 50% E.C.) manufactured by Devidayal (Sales) limited, India, was prepared using double distilled water. Serial dilutions of stock solutions were prepared using tap water as per dilution techniques (APHA, 2005). Static-with-renewal acute toxicity tests were conducted with ten fish in each graded concentration and 96 hours LC_{50} value was found to be 17.9 μgl^{-1} in a prior study. Three sub-lethal test concentrations *viz.*, 1.7, 0.17 and 0.017 μgl^{-1} were selected for inducing histological changes in fish liver. Ten fish for each concentration of test chemical were kept separately in three litres of toxicant treated media for 28 days. Food was given during the study period. Test water was renewed every 24 hrs. After 28 days of exposure, fish were sacrificed and intestine was removed immediately and kept in 10% Formalin, as fixative, for 24 h, dehydrated, embedded in paraffin and sections cut at 5 µm thickness and stained with Harris Haematoxylin and Eosin. Changes induced by treatment in the intestinal tissues were photographed and analyzed by light microscope at 10X eye piece magnification and 40X objective magnification {Olympus (model U-CMAD3) with Camera attachment of Samsung (model SDC-313B)}.

3. Results

The intestine of *Esomus danricus* 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. It merges with tunica propria of the underlying mucosal coat. The mucosa is raised into several longitudinal folds and the epithelial lining of mucosa consists of absorptive cells (Fig.1). 1.79 µg l^{-1} of Malathion administered intestine after 28 days of exposure showed distorted papillae with dense transmural chronic infiltration of inflammatory cell (lymphocytes). Ulceration and erosion of the mucosa along with vacuolation were also seen (Fig 2). 0.179 µg l^{-1} of malathion exposure for 28 days, on the other hand, showed inflammatory cells into full thickness of intestinal wall along with some vacuolation (Fig 3). In intestine of 0.0179 µg l^{-1} of malathion administration for similar duration of exposure, mild inflammatory cell (lymphocyte) infiltration in the lamina propria could be seen (Fig 4).

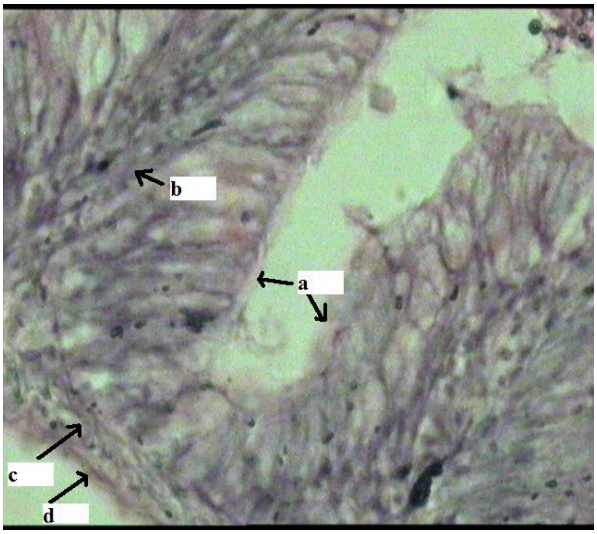

Fig 1: T.S of Control Intestine of *Esomus danricus*: (a) epithelium, (b) lamina propria, (c) muscularis, (d) serous membrane. H&E, 400×.

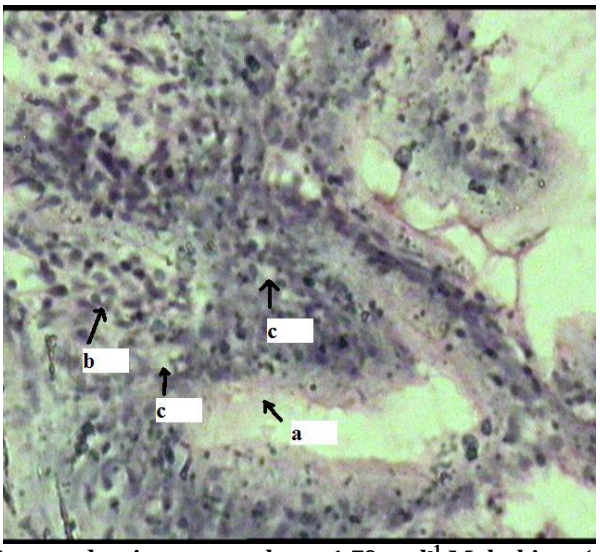

Fig 2: T.S of Intestine of *Esomus danricus* exposed to to1.79 µg l^{-1} Malathion: (a) ulceration of mucosa (b) infiltration of lymphocytes, (c) vacuolation. H&E, 400×.

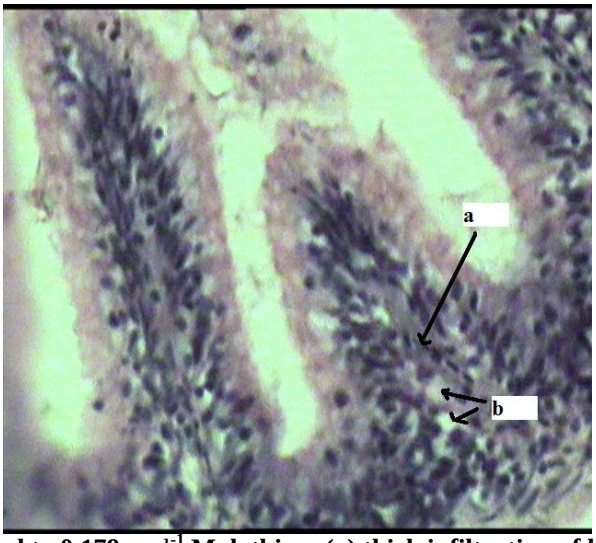

Fig 3: T.S of Intestine exposed to 0.179 µg l^{-1} Malathion: (a) thick infiltration of lymphocytes (b) vacuolation. H&E, 400×.

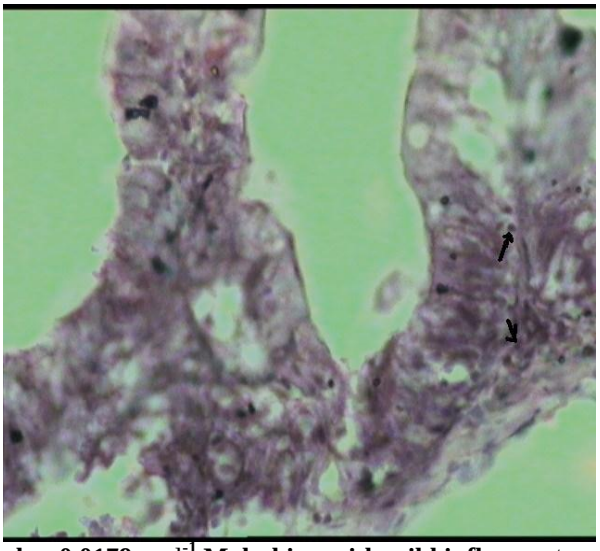

Fig 4: T.S of Intestine exposed to 0.0179 µg l^{-1} Malathion with mild inflammatory infiltration (↑). H&E, 400×.

4. Discussion

In the present study, the result of the effect of malathion on the gastrointestinal system of *Esomus danricus* clearly show that this pesticide exert toxic effects on the different layers of intestine. The alterations in the intestines of the flying barb were more severe in higher doses. In a study by Mohan (2003) on *Glossogobius giuris*, malathion was found to induce alterations both in the foregut and hindgut after exposing to each of 0.05, 0.25 and 0.5 ppm for 24, 48, 72 and 96-h of intervals. Malathion treatment resulted in breakdown and fragmentation of the serosal layer. In muscularis, the longitudinal muscle layer was greatly reduced and the circular muscle got thickened with an increase in the number of blood vessels. But in the present study, such changes could not be found. Rather, reactions like superficial erosion of mucosa, dense lamina propria and chronic inflammatory cell infiltration or transmural inflammation along with vacuolation were observed in the toxicant exposed fish intestine. 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). Epithelial degeneration, and inflammatory cells infiltration in the submucosal as well as submucosal edema were seen in the intestine of *Puntius gonionotus, Barbodes gonionotus and Clarias gariepinus* treated with diazinon and sumithion (Hoque et al. (1993); Lovely (1998). Velmurugan *et al* (2007) observed atrophy of epithelial cells, necrosis of epithelial cells, desquamation of mucosal epithelium and infiltration of lymphocytes into the lamina propria in the intestine of *Cirrhinus mrigala* exposed to fenvalerate. The present study also found profuse infiltration of lamina propria with lymphocytes, which is a mark of inflammation. According to Velmurugan et al. (2007), the changes observed in the different intestinal layers of the studied fish may be due to a direct effect of pesticides. The present results are in agreement with those observed by many investigators about the effects of different pesticides on fish intestine such as diazinon (Sakr, 1993); terbuthyjazine (Dezfuli *et al*, 2006); hexachlorocyclohexane (Das and Mukherjee, 2000); cyphenothrin (Erkmen *et al.*, 2000); thiodan (Cengiz *et al*, 2001); deltamethrin (Yildirim *et al,,* 2006); aldrin and heptachlor (Campagna *et al*, 2007); and fenvalerate (Velmurugan *et* al, 2007).

According to Desai *el al*. (1984), the degenerative and necrotic changes observed in the different intestinal layers of the studied fish may be due to a direct effect of the detected pesticides on the cells, to an accumulation of acetylcholine in the tissues or to a reduction in oxygen supply. According to Bhatnagar et al. (2007), the observed irritation and destruction of the mucosa membrane of the intestine hamper absorption. The pathological alterations in the intestine of the studied fish are in agreement with those observed by many investigators about the effects of different toxicants on fish intestine (Hanna et al. 2005; Cengiz and Unlu, 2006). Walsh and Ribelin (1975) reported hyperemia, degenerative changes in the tips of villi, loss of structural integrity of mucosal folds, hypertrophy vacuolation and necrosis in the intestine of *Cyprinus carpio* exposed to the pesticide atrazine. The present study, thus, concludes that although organophosphate pesticides are found to be less toxic to mammals than organochlorines, yet, very low doses of such pesticides (malathion) can cause apparent pathological changes in vital organs (intestinal) of non target organisms like fish.

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