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Short Communication

Histopathological changes in the Liver of Indian Flying Barb (*Esomus danricus)* exposed to Organochlorine Pesticide, Endosulfan (EC 35)

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Abstract

Indian flying barb (Esomus danricus) was exposed to sublethal concentrations of endosulfan (EC 35) for 28 days and liver histopathology was observed by light microscopy after staining with Haematoxylin-Eosine. The hepatocytes showed pathological changes ranging from mild inflammatory infiltration, swelling and vacuolation to nuclear picnosis and necrosis. Higher dose had more severe effect on liver.

Keywords: Hepatocyte, chronic, necrosis, vacuolation, picnosis.

Introduction

Endosulfan, an organochlorine pesticide, is used to control insects and mites infesting crops including vegetables, fruits, tea, coffee, cotton, rice and grains[1]. It is a neurotoxic pesticide that attacks the gamma aminobutyric acid receptor complex in the central nervous system[2]. Endosulfan is still used in many areas of the developing countries irrespective of ban or restricted use in many countries[3]. This fuels concern about potential pesticide run off into water bodies which harbours, amongst other, fishes. Endosulfan is partially degraded into endosulfan sulfate, which often enters the water bodies via surface runoff of contaminated soil. Endosulfan sulfate is highly toxic[4]. The Indian flying barb, *Esomus danricus* (Hamilton-Buchanan) inhabits shallow water bodies of Northern India and is a cyprinid that is economically important both as an ornamental and food fish. This fish runs the risk of ending up as the non target victims of such pesticides that are being sprayed in agricultural crops. Although liver plays crucial role in metabolism[5,] yet exposure of excess xenobiotics often cause structural changes of hepatocytes[6]. Endosulfan is reported to be hepatotoxic[7] and therefore, liver can serve as a potent biomarker for assessing endosulfan toxicity in fish[8]. The present study was thus, aimed to determine the histopathological effects of sublethal concentrations of endosulfan to Indian flying barb liver after 28 days of exposure.

Material 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[9]. 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. A stock solution of commercial grade endosulfan (Thiodan- endosulfan 35% EC, Bayer Crop Science, India) was prepared using double distilled water. Serial dilutions of stock solutions were prepared using tap water as per dilution techniques[10]. 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 4.9 μgl^{-1} in a prior study. Three sub-lethal test concentrations *viz*., 0.49, 0.049 and 0.0049 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 liver were 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 liver 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)}.

Results and Discussion

On analysis of control liver (figure 1) of *Esomus* at 400X magnification, a normal morphology with no pathological abnormalities was observed. The hepatocytes present a homogenous cytoplasm and a large central or subcentral spherical nucleus. The qualitative liver histology in fish exposed to 0.49μgl^{-1} endosulfan for 28 days showed inflammatory cell infiltration, picnotic nucleus, swelled hepatocytes and necrotic areas where hepatocytes have lost the membrane with dissolved nucleus (figure 2). In fish exposed to exposed to 0.049μgl^{-1} endosulfan for 28 days, the hepatocytes show inflammatory cell infiltration, picnotic nucleus, swelled hepatocytes and necrotic

areas (figure 3). In fish exposed to exposed to 0.0049µgl^{-1} endosulfan for similar duration, the hepatocytes show mild inflammatory infiltration, swelling with peripheral nucleus and necrotic areas (figure 4). In the liver, histological changes observed were more pronounced in fish exposed to higher endosulfan concentrations.

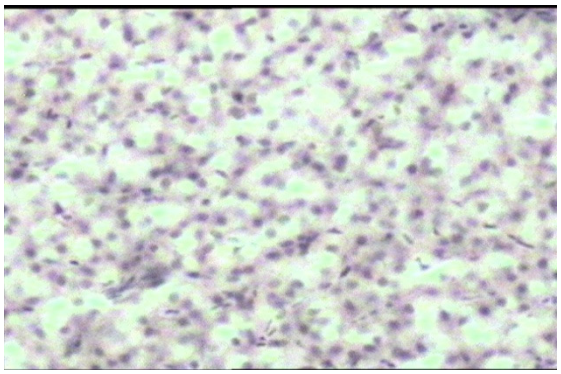

Figure-1
T.S. of control liver of *Esomus danricus*

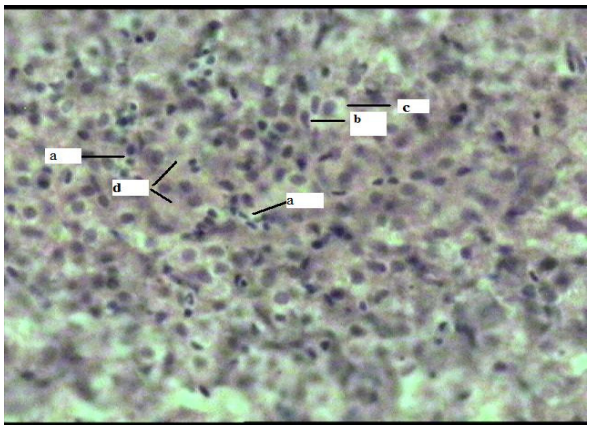

Figure-2
T.S. of liver of *Esomus danricus* exposed to 0.49 µgl^{-1} endosulfan (400X). The hepatocytes show inflammatory cell infiltration (a), picnotic nucleus (b), swelled hepatocytes (c) and necrotic areas (d)

Fish liver has the ability to detoxify pesticides but high concentrations of these compounds can alter hepatic enzyme activities that can result in damage of hepatocytes[11,12]. Several studies demonstrated that alterations in number, size and shape of the hepatocyte nucleus can be due to contaminants. In the present study, the flying barb hepatocytes show swelling and vacuolation, the nucleus is pushed towards periphery; some necrotic areas were also observed where hepatocytes have lost the membrane with dissolved nucleus. The swelling of hepatocytes can be indicative of increased activity of xenobiotic biotransformation enzymes as observed in perch, *Perca fluviatilis*[13] and sculpin, *Myoxocephalus scorpius*[14]. Alterations in size and shape of nucleus have often been regarded as signs of increased metabolic activity as well as pathological origin[15]. In the present study, higher doses of endosulfan caused picnosis of hepatocyte nucleus. Extensive degeneration of cytoplasm with piknosis of nuclei and loss of glycogen in liver tissue of *Heteropneustes fossilis* due to acute endosulfan (Thiodan) toxicity was also observed in another study[16]. In *Brachydanio rerio,* alterations in the size of nucleus have been reported to be influenced by contaminant[12]. The present study also showed that all the exposure doses caused inflammatory infiltration in the portal areas. Sublethal concentrations of endosulfan can cause behavioural disturbances and hepatic injury[17-18]. The liver histology in flying barb showed that endosulfan caused some alterations of the hepatocytes like vacuolization and necrosis. These alterations are often associated with a degenerative-necrotic condition[19]. In *Channa punctata*, endosulfan caused lipid peroxidation and disrupted antioxidant enzymes in liver[20] which in turn might lead to hepatic dysfunction. Besides, it appears that the degree of structural heterogeneity is enhanced with increasing concentration of the toxicant[21]. As seen in the present study, the liver histological changes observed were more evident in fish exposed to higher dose of endosulfan. Similar hepatic histopathological effects were observed in fish due to other chlorinated pesticides such as hexachlorocyclohexane [22] and lindane[23].

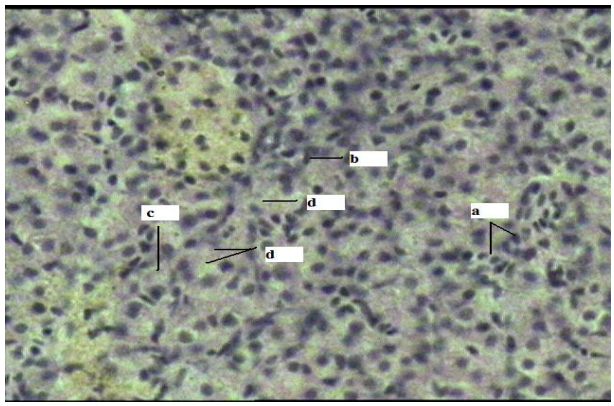

Figure-3
T.S. of liver of *Esomus danricus* exposed to 0.049 µgl^{-1} endosulfan (400X). The hepatocytes show inflammatory cell infiltration (a), picnotic nucleus (b), swelled hepatocytes (c) and necrotic areas (d)

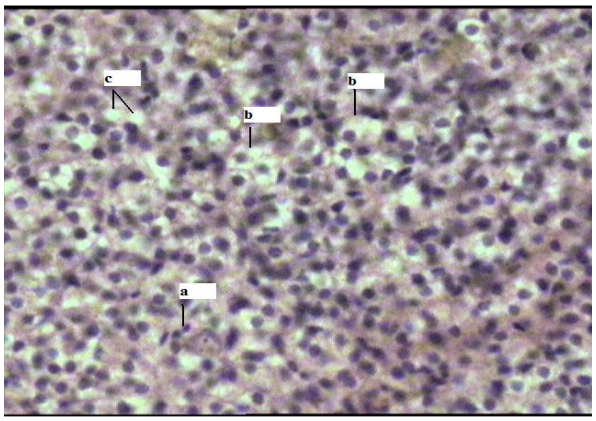

Figure-4
T.S. of liver of *Esomus danricus* exposed to 0.0049 µgl^{-1} endosulfan (400X). The hepatocytes show mild inflammatory infiltration (a), swelling with peripheral nucleus (b) and necrotic areas (c)

Conclusion

Thus, it is evident from this study that endosulfan, at very low doses, was able to induce pathological lesions in the liver of Indian flying barb.

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