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Research Article

HISTOPATHOLOGICAL ALTERATIONS IN HEPATOPANCREAS OF A CARP FISH, *C. CARPIO* DUE TO ENDOSULFAN TOXICITY

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ABSTRACT
Hepatopancreas occupies a central position due to various roles in fish. *Cyprinus carpio* is a fresh water teleostan found in paddy, little water reservoir that area prone to chemical deposition through break out mechanisms. Endosulfan is an organochlorine chemical, though illegal, however still used on varied crops. We tend to evaluate the histopathological effects of endosulfan on hepatopancreas of *C. carpio* using two totally different concentrations (0.0015ppm and zero.002ppm) for 10- and 20-days, each through accepted procedures of Mallory Triple and H and E Staining and located grume, hepatic vacuolations and nuclear atrophy, and complete degeneration of pancreatic cells showing that the duct gland is additional affected. Our results further substantiate the fact that endosulfan produces harmful effect within liver of fish and suggest that hepatopancreas could likewise be used as indicator chemicals toxicity in carp fish.

Keywords: *Hepatopancreas, Chemical, Endosulfan, Grume, Teleostan*

INTRODUCTION
Liver is a metabolic hub of activities performing most of the catabolic, anabolic, and detoxifying processes besides recycling of nutrients and maintaining minerals balance, and thence occupies a central position within the life of hepatopancreas having fishes. Moreover, liver performs hematopoiesis in adult fish and amphibian. In some fishes, liver is found en masse pancreas as hepatopancreas. Pesticides, which are requisite weapons of farmers, are used to increase either food production or to control different types of insects. Endosulfan is an organochlorine chemical, used on varied crops, most often, due to low cost. But, several countries have illegitimate it because of its varied adverse consequences on entirely diverse aquatic and non-aquatic and target or non-target organisms. Endosulfan has been classified as an extremely detrimental chemical (IFCS 2003 The Intergovernmental Forum on Chemical Safety). Regarding twenty thousand individuals are killed due to accidental poisoning. A number of works attested the harmful effect of endosulfan on totally different animals. In vitro, endosulfan mars respiration within the liver mitochondria of rat, (Dubey *et al.,* 1984), rainbow trout and catfish (Mishra and Shukla, 1994) and in vivo (Mishra and Shukla 1994; Arnold *et al.,*1996); macrophagic invasion, and buildup of myelinated bodies within the endothelial cells of sinusoids of liver at ultralow doses (Braunbeck *et al.,* 1999); sub-chronic exposure to EDS produces alterations in the somatic indices of liver apart from aerophilic stress in carp (Salvo *et al.,* 2012); endosulfan together with methyl group insecticide(methyl parathion) causes modifications in the behavior of adult rat (Castilo *et al.,* 2002). This study is dole out to check histopathological sensitivity of the hepatopancreas of *C. carpio* to sublethal doses of zero.0015-ppm and zero.002-ppm endosulfan concentration.

MATERIALS AND METHODS
Fishes were collected from fish farm of Raigarh within the Gregorian calendar month before the beginning of the rainy season employing fish web and brought to the laboratory in aquria where initial treatment with zero.01% $KMnO_4$ solution ready in farm water was given for fifteen minutes; they were retained for fifteen days in fibre aquaria for acclimatization. Raigarh is set at 21.9°N and 83.4°E (falling rain genomics 1996-2010). The Kelo stream divides the town of Raigarh into two parts. The fish farm is

found at a distance of one kilometer from the stream. Experiment was dispensed throughout the season. The chemical endosulfan was bought from the market of Raigarh. It was factory-made absolutely thirty fifth EC of Excel Industries Ltd. Bombay. Treatment solutions of zero.0015ppm and zero.002ppm were created in acetone having a toxicity of twelve thousand parts per million for fish, and regarded as harmless. The experimental solution was left for twenty-four hours as it is. Three water aquaria with a capacity of two hundred liter were taken. One aquarium had dechlorinated water and the remaining two contained EDS solutions of zero.0015ppm and zero.002 part per million. The parts per million solutions were prepared by serial dilution technique. Fishes that measures ten to fifteen cm and weigh close to sixty gms, were placed in the aquaria. We maintained oxygen supply through aerator throughout the experimentation period. 5 fishes from each aquarium were sacrificed after 10 days and the remainder of the fishes following twenty days. The fishes were fed with normal fish food daily. The liver of the sacrificed fishes was taken, cut into six to eight micron thick sections, and washed in normal saline water. Some pieces were fixed in Zenker's fluid (for Mallory triple stain), whereas some were fixed in Bouins fluid (for H and E), and are subjected to sectioning and staining. Temperature, pH, dissolved gas (DO), free carbon dioxide, alkalinity, hardness of water (temporary and permanent), and chloride were checked on an interval of 5 days by normal ways and found to be within the counseled range for carp rearing.

RESULT

Histopathology of the liver of untreated Cyprinus carpio communis

The Histological study of the Liver of the control *Cyprinus carpio communis* showed that the polyhedral hepatic cells are arranged in chains of which some of them are organized into a group of 4 – 6 cells, bound by very thin membrane. These hepatic cells containing centrally placed nucleus show prominent nucleoli, granular cytoplasm, but certain cells have peripheral cytoplasm too. Lobules of the hepatic cells exhibit no definite pattern of arrangement and the phagocytic cells are visible in between the lobules. Few blood spaces of varying size can be seen scattered throughout the liver without any definite shape. The hepatic ducts appear much larger than the bile ductules. Pancreatic mass, situated around the branches of hepatic portal veins, diffuses into the hepatic cells. A space can be observed in between hepatic and pancreatic cells. The pancreatic mass consists of two parts, i.e., exocrine and endocrine parts. Exocrine cells are larger and elongated, while endocrine cells are smaller and round. Exocrine cells are arranged in an acinus form with distinct nucleus, while endocrine cells are scattered in between the hepatic portal veins and the exocrine pancreas. Some of the endocrine parts of the pancreatic tissue consist of beta cells, which are round in shape with no clear distinction, while alpha cells are prominent and smaller. Blood vessels and blood spaces show prominent red blood cells (Figure 1, 2).

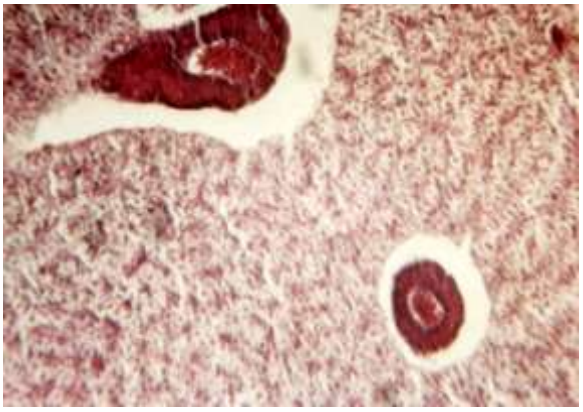

Figure 1: T S of liver of *C. carpio* H and E X-100.

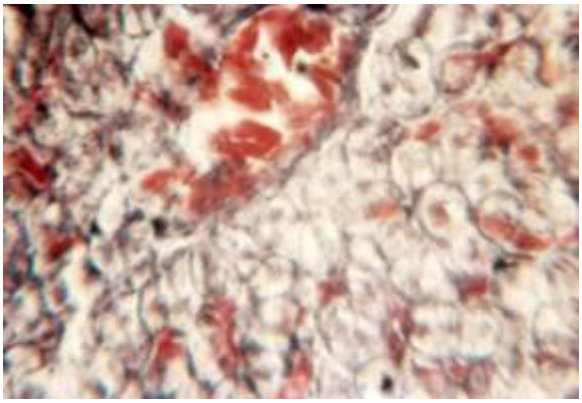

Figure 2: T S of liver of untreated *c.carpio* (Mallory Triple Stain) X-400.

Histopathology of the liver of treated Cyprinus carpio communis

EDS 0.0015 ppm intoxication

Histopathological examination of fish liver treated with 0.0015-ppm endosulfan for 10 days showed ruptures between hepatic cords; delimitation of the cell membrane of hepatic cells and connective tissues; a large blood space besides complete separation of blood vessels from the hepatic cells at some places; larger hepatic ducts and bile ductules as compared to control one; reduced pancreatic tissue with indistinguishable exocrine and endocrine parts that might result from pancreatic necrosis; broken junction between the hepatic and the pancreatic tissues along with clear empty space (*Figure* 3). In 20 days exposure liver showed formation of numerous tiny spaces between the hepatic cords, complete destruction of connective tissues at some places, clear atrophy in hepatic cell nucleus and homogeneity in hepatic mass. Pancreatic tissues are shrunken and reduced, resulting in a loss of differentiation between the exocrine and the endocrine part. At the junction of hepatic and pancreatic tissue, a clear vacuolization is observed which may be the result of necrosis in hepatic tissues (*Figure* 4).

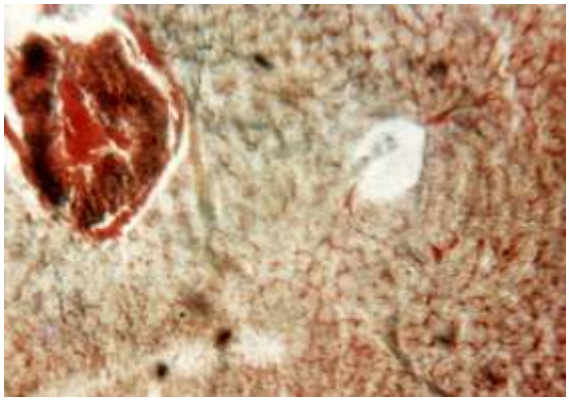

***Figure* 3: T S of liver of *C. carpio* after 10 days exposure to 0.0015ppm EDS (Mallory Triple Stain) X-100.**

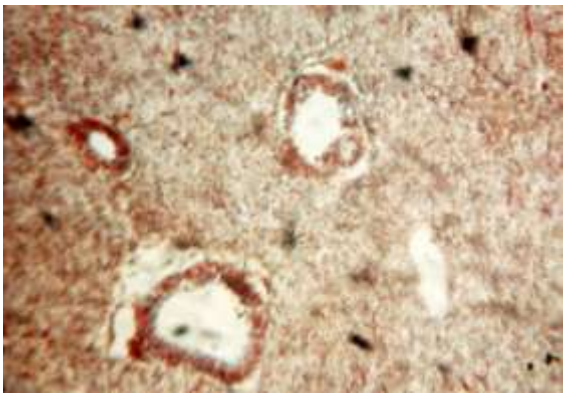

***Figure* 4: T S of liver of *C. carpio* after 20 days exposure to 0.0015ppm EDS (Mallory Triple Stain) X-100.**

EDS 0.002 ppm intoxication

Following treatment with 0.002-ppm EDS for a period of 10days, the liver of *Cyprinus carpio* showed a large number of vacuoles in and around the hepatic cells providing spongy appearance in certain parts of the liver. Hepatic cells show heavy necrosis and damage as breakings.

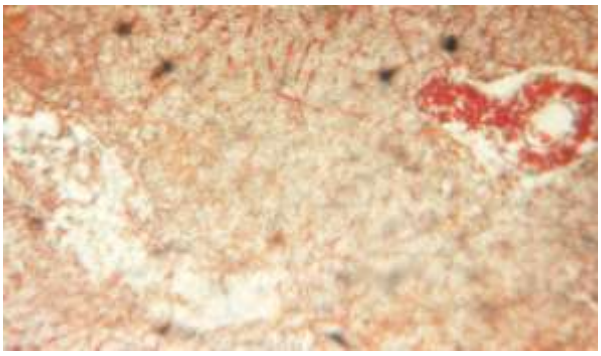

***Figure* 5: T S of liver of *C. carpio* after 10 days exposure to 0.002ppm EDS (Mallory Triple Stain) X-100.**

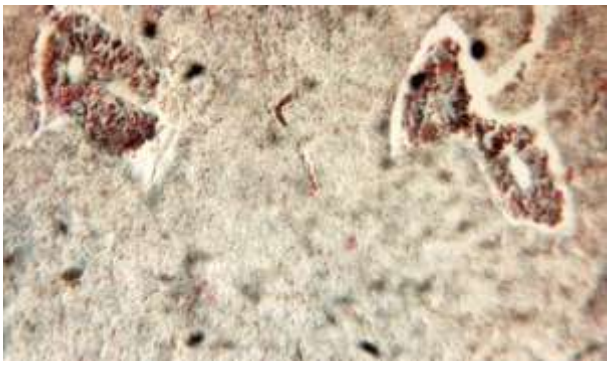

***Figure* 6: T S of liver of *C. carpio* after 20 days exposure to 0.002ppm EDS (Mallory Triple Stain) X-100.**

Outer wall of the blood vessels reduced in thickness, while in some cases it was found in broken condition. Large spaces appeared around the blood vessels and pancreatic mass due to necrosis in hepatic tissues. Pancreatic mass appeared in shrunken condition (*Figure* 5). In response to 20 days exposure liver showed similar histopathology as that of 10 days exposure except vacuolations in and around the hepatic cells increased heavy necrosis. Pancreatic tissue showed strong homogeneity at some places (*Figure* 6) besides exhibiting decrease in number of exocrine tissue.

Hence, we opine that the pancreas is more affected by the sublethal doses of endosulfan as complete destruction of pancreatic mass is observed. Vacuolations in hepatic cells increase with dose and time.

DISCUSSION

Salvo *et al.,* (2007) also found breakoff point in hepatic cell borderline and agglomeration at 0.001mg/L of EDS in *Cyprinus carpio*. Raj *et al.,* (2013) reported hemorrhage in winster rat liver that was treated with a mixture of endosulfan and cypermethrin (207.50 mg/kg BW). Within the craniate foetal liver of winster rat, vacuolar degeneration, enlargement of nucleus associated severe curving dilation at an oral dose of one mg per kg of body weight of endosulfan had additionally been shown (Singh *et al.,* 2008). Chan and Mohd (2005) have found endosulfan sulphate within the liver of rats in the range of zero.02-0.22 µg g^{-1}. Matthiessen and Roberts (1982) had reported gangrene, focal gangrene, and subcapsular hydrops within the liver of *C. gariepinus* at an ultra–low–volume doses (6–12 g/ha) of endosulfan, however, they had reported the disappearance of endosulfan before the ending of spraying season. In *Chana punctatus*, Sarma *et al.,* (2012) had pointed out severe alterations in liver. In *Labio rohita*, endosulfan exposure produces vacuolization in hepatocyte (Neeraj *et al.,* 2012). Concentration independent histopahological lesions were also discovered in liver, gills, spleen and trunk kidney in response to endosulfan (Altinok and Capkin, 2007), however, distinction is that they discovered reversible changes whereas in our experiment alterations were irreversible that increased with time. In *Channa punctatus* at a sublethal endosulfan concentration (8.1 microg l(-1)), after ninety six hour brain showed delicate gangrene within the apical lobe of the cerebral hemisphere, the sole part they examined (Sarma *et al.,* 2010). Reversible microscopic modifications (like hydropic degeneration and sinusoidal dilation) were also discovered within the liver of exposed fish besides an irreversible modification like gangrene at the highest concentrations (Ballesteros *et al.,* 2007).Thus, our works substantiate the findings of different researchers that endosulfan produces changes in internal organs.

Conclusion

Our study concludes that endosulfan produces harmful effects even at 0.0015- and 0.002-ppm concentration. Hence, it should be replaced by some ecofriendly biopesticide.

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