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# EFFECT OF BHC ON HISTOPATHOLOGICAL CHANGES IN LIVER OF *CYPRINUS CARPIO*

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## ABSTRACT

*Cyprinus carpio* was exposed to BHC for the period of 10 to 20 days. After BHC exposed fishes were sacrificed for microscopic study between control and treatment. Result indicate that 1.5ppm and 2.5ppm BHC intoxication, liver showed vacuolization, swelling of the hepatocytes with diffuse necrosis, separation of blood vessels from hepatic cells in comparison to control. Present study indicate that the BHC is very harmful for the aquatic system. It produced various alteration in the different organs of aquatic organism.

## INTRODUCTION

Pesticides are used on large scale to improve the production of food grains. Pesticides are used to kill and keep away the unwanted and harmful insects & pests. Through pesticides are used for better production of crop but at the same time these pesticides also enter in ponds and lakes through rainwater. It affects the life of living things of water mainly fishes. On mixing with water, it also changes the chemical composition of water & makes it harmful for animals that live in water. Since fishes are fully dependent on water & breath under water to keep them alive. These chemicals enter into fishes' body and bring changes in internal structure of the organs slowly and slowly. These fishes are very sensitive & brings the effect of pesticides very fast even after very small quantity. Its mortality rate is very high. These pesticides affect the

tissue of fishes including *Cyprinus carpio* & due to this many organs pass through the histological change.

Human population explosion causes an increase in pressure on world agriculture to produce more food grains in order to meet the augmented food requirements. This has led to the development of high yielding varieties to increase the crop productivity, but these high yielding varieties were more susceptible to diseases and pests in comparison to old varieties. To overcome this problem, pesticides of various types were developed, though these chemical are evolved to kill and reduce the crop productivity yet they are also toxic to the economic form of life or non target species.

The aquatic life is constantly threatened by the seepage of pesticides and their constituents from agricultural fields, industrial and domestic sewage. Fishes are much vulnerable to toxic substances and their bioaccumulation cause serious risk to life. Such toxic substances and their bioaccumulation cause serious risk to life. Such toxic substances enter to human through food chain, as fishes constitute an important part of animal protein in rural and Urban areas. Their availability and selectivity to toxic substances are main criteria for selection as an experimental animal.

## MATERIAL AND METHODS

Live specimen of *Cyprinus corpio* were collected from the Kulipota fish farm. These fishes were treated with 0.1% potassium permagnate solution for 15 minute to get rid of dermal infections. All the fishes were kept for acclimatization in polymer fiber aquaria for about 7 days. Healthy fishes, measuring about 10-12 cm and weighing approximately 40-50 gms were selected for experimentation. A set of 10 fishes were transferred into three different maintained polymer fibre aquaria with 200liter water quantity, out of which one contained dechlorinated water and rest of the two contained 3ppm and 5ppm concentration of BHC. Five fishes were sacrificed after 10 days and rest of fishes were sacrifies after 20 days. All the fishes were feed daily the tenure of experimentation. The liver of sacrificed fish is taken out, cutted in small pieces and the pieces were treated with normal saline to remove the blood. Then the pieces of liver were fixed in aqueous bouin's 10% neutral formalin and Zenker's fluid, block preparation, sectioning, staining, and examining under light microscope for histological changes.

## RESULTS

### 1. Histology of the liver of untreated *Cyprinus carpio communis*

The hepatic cells were polyhedral in shape and arranged in chain but some of them were arranged in groups of 4-6 cells which were bound by very thin membrane. These cells contained centrally placed nucleus, which also showed prominent nucleoli. Cytoplasm of the hepatic cells was granulated but in certain cells, it was found only at the peripheral part of the cells. Lobules of the hepatic cells have no definite pattern of arrangement. The phagocytic cells were visible in between the lobules. Few blood spaces of varying size were found to be scattered throughout the liver mass. They had no permanent shape. The hepatic ducts appeared much larger than the bile ductless.

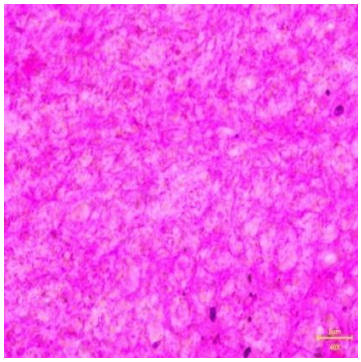

**Fig.1**

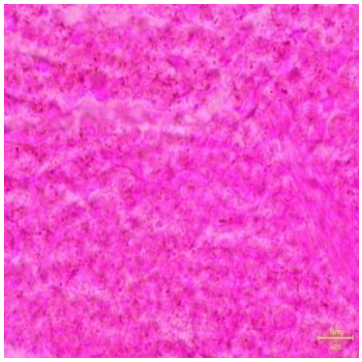

**Fig.2**

**Fig.1- Photomicrogram of transverse section of liver 10days, untreated *C.carpio* (hematoxylin and eosin stain) X-400.**

**Fig.2- Photomicrogram of transverse section of liver 20days, untreated *C. carpio* (hematoxylin and eosin stain) X-400.**

Hepatic cells were transversed by diffused pancreatic mass. The pancreatic mass was situated around the branches of hepatic portal veins. A gap was found in between the hepatic and the pancreatic cells in general. It seems that the pancreatic mass was attached to the hepatic mass by some invisible very thin membranous structure.

The pancreatic mass was made up of two parts, i.e., exocrine and endocrine parts. Exocrine cells were larger and elongated, while endocrine cells were smaller and rounded in size. Exocrine cells were arranged in an acinus form with distinct nucleus, while endocrine cells were scattered in between the hepatic portal veins and the exocrine pancreas. Some of the endocrine parts were also present in between the exocrine cells. The endocrine parts of the pancreatic tissues consists of beta cells, which were rounded in shape with no clear nucleus

distinction, while alpha-cells were prominent but they were smaller in shape. The blood vessels and blood spaces showed prominent presence of red blood.

**2. Histopathology of the liver of treated *Cyprinus carpio communis***

**BHC 1.5ppm intoxication**

In the present investigation, BHC intoxication (1.5ppm BHC for 10 days), it was observed that small spaces appeared in between hepatic cords, nucleus displaced slightly, large connective tissues and cell membrane of the hepatic cells broke and complete separation of blood vessels from hepatic cells besides reduction of pancreatic tissue were found. There by leaving a very narrow opening of the hepatic portal vein. Twenty days exposure caused formation of so many small spaces between hepatic cords, connective tissues destructed completely and atrophy of nucleus in hepatic cells, no further changes were seen in the cytoplasm of the hepatic cells. Pancreatic tissues shrank obliterating the differentiation between the exocrine and endocrine pancreas.

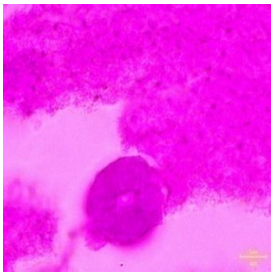

**Fig.3**

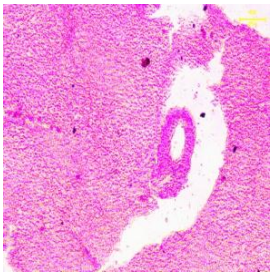

**Fig.4**

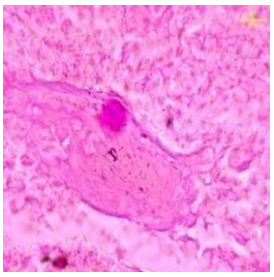

**Fig.5**

**Fig. 3- Photomicrogram of transverse section of liver 10 days, 1.5 ppm *C. carpio* (hematoxylin and eosin stain) X-400.**

**Fig. 4- Photomicrogram of transverse section of liver 20 days, 1.5 ppm *C. carpio* (hematoxylin and eosin stain) X-400.**

**Fig. 5- Photomicrogram of transverse section of liver 10 days, 1.5 ppm *C. carpio* (hematoxylin and eosin stain) X-100.**

**B) BHC 2.5ppm intoxication**

The tissue treated with 2.5 ppm BHC for 10 days showed a large number of vacuoles in between hepatic cells, liver converted into spongy mass, heavy necrosis in the hepatic cells, outer wall of blood vessels reduced in thickness while in some cases it broke. Large spaces appeared around the blood vessels and the pancreatic mass. Pancreatic mass appeared in

shrunken condition. No further variation was seen after twenty days exposure except vacuolation in and around hepatic cells increased.

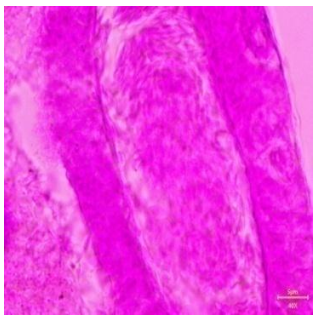

**Fig.6**

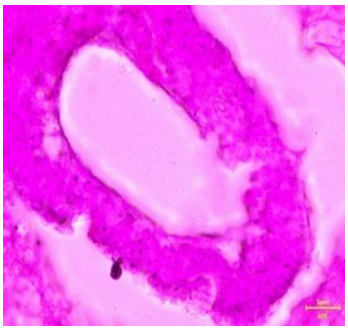

**Fig.7**

**Fig. 6- Photomicrogram of transverse section of liver 10 days, 2.5 ppm C. carpio (hematoxylin and eosin stain) X-400.**

**Fig. 7- Photomicrogram of transverse section of liver 20 days, 2.5 ppm *C. carpio* (hematoxylin and eosin stain) X-400.**

## DISCUSSION

Present study of *Cyprinus corpio* in the liver pancreatic tissues are diffused in between the hepatic cells. Diffused pancreas have also been reported by several workers like smallwood, & Derrickson (1934) in *Labeo calbasu*. Kasotia (1988) in *Channa gachua*. Mishra (1988) in *Glossogobius giuris*.

Liver of fish observed the polyhedral shape of hepatic cell with centrally placed nucleat and granulated. Liver mass was traversed by the hepatic duct and pancreas by the branch of hepatic portal veins but the liver cords were not prominent. Fish liver also observed the pancreatic cells in the acinus form having exocrine and endocrine pancreatic cells, above findings are very much in conformity with the findings of king (1962) in the guppies and groud trout, Konar (1965), in *Labeo rohita*, Kaur *et al.* (1977) in Mystus tengra, Kasotia (1988) in *Channa gachua*, Jha (1990), *Channa punctatus*. Arora and Kulshrestha (1984) studied the effects of chronic exposures to sublethal concentration of two pesticides Carbaryl and Endosulfan on alkaline and acid phosphatase activities in the intestine of a fresh water teleost *Channa striatus*. The intake of insecticides affects the biochemical composition of fishes (Jebakumar *et al*., (1990); Sultatos, 1998; Kumble and Muley, 2000; Prasad *et al*., 2002). It has been shown by many scientists that insecticides mainly affect liver of fishes (Murty and Devi, 1982; Anthony et al., 1986; Bhushan et al.2002). This is because of its

relatively slow blood flow as compared to cardiac output (Gingerich, 1982) as well as the much closer association of hepatocytes to biliary system than is found in mammals (Hinton and Lauren, 1990) Jyothi B, Narayan G (2004), observed liver damage, K.V. Sastrey and Kamana Sharma (2004), observed in the liver after 15 days were liver cord disarray, rupture of cell membrane, vacuolation in the cytoplasm of hepatocytes, and enlargement of nuclei. The liver damage became more severe in fish exposed for 30 days and was characterized by fibrosis, small degenerated nuclei, and invasion of a number of phagocytes into the necrosed tissue.

D.P. Jaroli and B.L. Sharma (2005), Dixit Yogesh Babu, Saxena KK, Chauhan Shalini, Dubey AK (2005), observed that the protein content decreased, whereas the specific activities of acid phosphates (ACP) and succinate dehydrogenage (SDH) showed an increased initially in the liver of catfish, *Heteropneustes fossilis* exposed to rogor. Sinha Dharmendra Kumar, Verma P, Kumar A, Nath A (2005), observed various damage, degeneration and toxicity caused to the pesticide induced cells. The increasing hepatic lesions may serve as bioindicators of exposure to environmental contaminants. It is possible that increased autolysis of hepatic cell could ultimately led to cell death and also affect other organs. Mehrotra and histochemical changes in intestine of freshwater fish *Nandus nandus*. were exposed to Endosufan (0.04) for one month. S.R. Pugazhvendan, N. jothi Narendiran, G. Kumaran, S. Kumaran and K.M. Alagappan (2009) observed degenerative changes included hypertrophy of cells and their nuclei, liver on the whole showed distance appearance. Pycnosis, Vacuolation, Necrosis in Ophiocephalus puctatus exposed to Malathion.

In the present investigation in the liver of *Cyprinus corpio* after 10 days intoxicationo of 1.5 ppm BHC showed slight change and nucleus and broken hepatic cells but after 20 days exposure vacuolation and necrosis was observed in the hepatic cells in the intoxication of 2.5 ppm BHC after 10 days showed vacuolization in the hepatic cells, heavy necrosis was noted in the hepatic cells and some hepatic cells showed broken cell wall, smaller damages were also after 20 days exposure.

These results are very much similar to the results of above cited workers.K.V. Sastry and Kamana Sharma (2004), observed Histopathological Changes in liver, were liver cord disarray, rupture of cell membrane, vacuolation in the cytoplasm of hepatocytes, and enlargement of nuclei. The liver damage became more severe in fish exposed for 30 days and was characterized by fibrosis, small degenerated binucleated nuclei, and invasion of a number

of phagocytes into the necrosed tissue in teleost fish, Ophiocephalus punctatus exposed to two sublethal concentrations of diazinon (0.2 and 0.4 mg/liter). Degenerative changes included hypertrophy of cells and their nuclei of liver, whole showed distance appearance, binucleation, Pycnosis, Vacuolation, Necrosis were observed by S.R. Pugazhvendan, N. Jyothi Narendiran, G.Kumaran, S. Kuamaran and K.M. Alagappan (2009), after Malathion intoxication, similar results were obtained during present investigation the liver of *Cyprinus corpio* after the exposure of BHC.

In the present study after BHC (0.0015 ppm) intoxication large spaces were in between the hepatic cord and cell wall of the hepatic cell were found in broken condition. In longer duration the connective tissue were found completely destructed at some places, atrophy and vacuolation in hepatic cells. In higher concentration heavy vacuolization was seen in the hepatic cell and due to heavy necrotic activities some of the hepatic cells showed broken cell wall. Longer duration heavy necrosis was seen in the liver mass.

Ratnakar and Awasthy (1979) reported vacuolation and slight change in hepatic cells with low concentration of Aldrin in the liver of *Cyprinus corpio* while in higher concentration. They observed the degeneration of liver cells. They also observed deformation in the shape of hepatic cells. Similar observation were made by Sastrey and Sharma (1979). Mandal & Kulshrestha (1980) and Mc Brridge (1981) in the liver of *Channa puctatus*, *Clarias batrachus* and Oncorychus mercarespsctively after pesticidal exposures. Shrivastava and Shrivastava (1982) reported hypertrophy of nucleus vacuolation in the hepatic tissue due to Shrinkage in cytoplasm, disruption of cynocides in the liver of *Cirrhina mrigala* after urea intoxication Konar (1983) in Labeo et al. (1983) and Rashatwar & Ilyas (1984) in *Clarias batrachus* and *Nemacheilus denisonii* respectively.

Rashmi & Mishra (1994) reported pathological lesion in the liver of *Puntius ticto* after herbicides Helvozine intoxication they have observed hypertrophy of hepatocytes disorientation of liver cord and vacuolar degeneration of cytoplasm. Bhatnagar et al. (1994) also observed similar destruction in the liver of *Clarias batrachus* after Malathion intoxication, hypertrophy of hepatic cell and necrosis were also reported by Shinizu and Akira (1994) in the line of Pagrus major after bis (Fri- ra-buty line) oxides (TBTO) exposure. Anita and kumar (1995) worked on the effect of industrial effluent of the liver of Heteropneustes, fossilis and reported the histopathological changes in the liver. Histology of liver showed degeneration, necrosis, vacuolation of hepatocytes and rupture of nuclear and

cell membrane. David M, Shivkumar HB, Shivkumar R, Mushigeri SB, Ganti BH (2003), studied Toxicity evaluation of cypermethrin and its effect on oxygen consumption of the freashwater fish *Tilapia mossambica*. Jyothi B, Narayan G (2004), studied serum cholinesterase levels in fish *Clarias batrachus* (Linn.) exposed to pesticides carbaryl and phorate. Maruthanayagam C, Sharmila G (2004) studied Haemato-biochemical variations induced by the pesticides monocrotophos in *Cyprinus carpio* during the exposure and recovery periods.

The present investigation after 10 days exposure of 1.5 ppm BHC intoxication, pancreatic tissues were seen in reduced condition due to necrosis in the pancreatic cells it became difficult to identify to exocrine and endocrine parts. Attachment between the hepatic and pancreatic tissues was broken at some places was seen. No further deterioration was seen after 20 days exposure in higher dose (2.5 ppm) intoxication pancreatic mass appeared in shrunken condition.

## CONCLUSION

Liver is one of the important organ that detoxify harmful chemicals and it under goes such a tremendous alterations such as death of hepatic cells, vacuole formation, destruction of pancreas etc. the liver are the target organ to be affected. Liver showed extensive damage due to added burden of insecticide detoxification. In response to the exposure of the pesticide (BHC) which will not only reduce the quality of fish but also affect other organism and of course human being is no exception. Therefore, the use of the pesticide should be diminished and other sources, which are less harmful or harmless should be explored.

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