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HISTOPATHOLOGICAL STUDIES ON LIVER AND KIDNEY OF *CHANNA PUNCTATUS* (BLOCH) EXPOSED TO MANCOZEB CONTAINING PESTICIDE DITHANE M-45

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ABSTRACT

Indiscriminate use of pesticides in agricultural fields affects a wide range of non target organisms including fish fauna. The present studies deals with the impact of Dithane M-45 (80% mancozeb) on histology of liver and kidney of *Channa punctatus*. After acclimatization in the laboratory condition, fishes are divided into control and treated groups. Exposure of the fish, *C. puntatus* to sublethal concentration (3 ppm) of Dithane M-45 over a period of 30 days has revealed that the pesticide had a profound effect on target organs with remarkable histological changes.

KEY WORDS : Dithane M-45, *Channa punctatus*, Histopathology, Liver, Kidney.

INTRODUCTION

A number of human activities pollute the aquatic environment. Now-a-days various types of insecticides, herbicides, fungicides and fertilizers have been used by farmers to control the target species of pest and increase the crop production. Due to raining or irrigation the runoff water carries the pesticides from the agricultural fields into the nearby fresh water reservoir, leads to toxic pollution in aqua bodies (Mason, 1991). These toxic substances affect a wide range of non target organisms such as invertebrates and fishes inhabiting aquatic environment (Pandey, 1988). The increasing use of pesticides in agricultural fields leads the natural imbalance, decrease soil and water quality. The degraded or break down product of pesticides also causes significant toxicological risks to resident organisms (Kumari and Kumar, 1997). Pollution caused by the pesticides severely affects aquatic organisms along with the entire food chain including human beings (Dutta and Maxwell, 2003). Aquatic organisms accumulate toxic substance directly from the contaminated water and indirectly through the food chain. These pesticides can directly kill or affect the zooplanktons which are the major food of fishes. The fish species may be affected on being eating those zooplanktons. In another way the chemical pesticides mixed in water may be directly affect respiratory organs like gills. When such xenobotic compounds enter the food chain of aquatic ecosystem they affect human health too via ecological cycling and biomagnification (Sun *et al.* 2000). It has also been recorded that such toxic chemicals can incorporate even at low concentration in the vital tissues of fishes and birds with serious alterations. (Chakraburthy and Konar, 1974; Mathur *et al.* 1981). Dithane M-45 is a fungicide widely used in vegetables and cereals crops especially in rice field crops. This pesticide contains 80% Mancozeb which is a contact fungicide, a subclass of carbamate pesticides called dithiocarbamates. The dithiocarbamate is analogous to carbamate in which both oxygen atoms are replaced by sulfur atoms. Mancozeb is combination of two fungicides/ compounds maneb (manganese) and zineb (zinc ion) (Hayes and Laws, 1991). Mancozeb has been shown to produce adverse effects on skin, liver, kidney, central nervous system (CNS), male and female reproductive system or chromosomes of bone marrow cells in Mice, Rat and Human (Anantham and Kumaran, 2013). Histological investigations have proved to be an important tool to detect direct effects of chemical compounds on

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specific organ (EIFAC, 1983). A number of studies have demonstrated the toxic effect of various pesticides on different fish species and few of them are- , histopathology of *Labeo rohita* exposed to sub-lethal doses of hexachlorocyclohexane (Das and Mukharjee, 2000), Rainbow Trout exposed to mancozeb (Muhammed and Telat, 2003), pesticides accumulation in fish body tissue of *Channa punctatus* (Ramesh and Maheswari, 2004), histopathological changes in the gill, liver, brain and kidney of the Indian major carp *Cirrhinus mrigala* exposed to chloropyrifos (Tilak *et al.,* 2005); *Channa punctatus* exposed to carbosulphan (Nwani *et al.* 2010), Genotoxic and haematological studies (Shahi and Singh, 2014). But, still there is paucity of information regarding the long term exposure of pesticides of sublethal concentration on histology of target organs. Hence the present work was aimed to find out the impact of single dose of sublethal concentration of Dithane M-45 on histological studies on target organs of *Channa punctatus.*

MATERIALS AND METHODS

Freshwater water fish *Channa punctatus* was collected from a beel of Kalpani (village) area of Baksa district with the help of a fine net. The fishes were measuring 8-20 cm and weighs about 30-80 gm. 30 fishes were collected and brought to the laboratory in plastic container. The experiments were done at laboratory of Zoology Department, Bajali College, Pathsala (Assam). The fishes were then washed in the running tap water and then put to deep treatment in 0.1% $KMnO_4$ (Potassium permanganate solution) for 10 minutes to prevent the dermal infection during the time of acclimatization period. The fishes were then kept for acclimatization for 7 days in two glass aquarium of 50 liter capacity–one as control group and the other as treated group. During the time of acclimatization period the fishes were feed with artificial food available in the markets.

Toxicity level determination

Dithane M-45 was (Dow Agrosciences) obtained from the local market whose composition is Mancozeb 80% and Balance 20%. To investigate the 96hr LC50 values of toxicity level, the fishes were treated with five different concentrations of Dithane M-45 were prepared by dissolving in distilled water. Five different concentrations of Dithane M-45 were used to find out 96hr LC50 which were in the range of 4 to 12 ppm (4 ppm, 6 ppm, 8 ppm, 10 ppm and 12 ppm). Mortality was recorded for each day and percentages of mortality at the end of 96hr for the different groups were recorded. 50% mortality was recorded in the 10 ppm group. Finally the 96hr LC50 value was determined using online computer programme based on the Finney *et al.* (1971) probit analysis method and recorded as 9.5 ppm. Approximately one third of LC50 values obtained from this experiment (3ppm) was taken as the sub lethal concentration for the present investigation. During the time of experiment both the control and treated fishes were fed with same food as in the acclimatization period. Both the fish groups were kept for 30 days. Histological slides were prepared in two different periods, one after 15 days and finally after 30 days. For this the fishes from both the control and treated were taken out and carefully dissected out the liver and kidneys. Fixation was done in Bouin's Fluid. After washing and dehydration, the tissue was embedded in paraffin wax. Thereafter the paraffin blocks were trimmed and sections are cut at 4 microns with the help of rotatory microtomes. The double staining methods were applied using Hematoxylin and Eosin stain. Slides are then mounted with D.P.X. and observed under microscope and photomicrographs were taken at 400X.

RESULTS AND DISCUSSION

Liver: The histological structures of the liver of control fish showed normal hepatocyte cells, parenchymal cells and sinusoids. Hepatic cells are roundish polygonal containing nucleus. The kupffer cells are attached to the walls of sinusoids and show the hepatic acini (Fig. 1.a).

Several histopathological alterations were observed in the liver of Dithane M-45 treated fish. The observed changes are degeneration of hepatic tissues, and formation of vacuoles was seen after the treatment of 15 days and 30 days. The degeneration of hepatic cells is due to necrosis of hepatic cells was prominanatly observed after treatment of 30 days (Fig. 1. b,c).

Pesticides found to have causes profound histopathological changes in liver of different fishes and reported by various authors. Desai *et al.* (1984) reported that histopathological changes in the liver of *Tilapia mossambica* after the treatment of organophosplate monocrotophos. He reported that vacuolation of hepatic cells and coagulation of

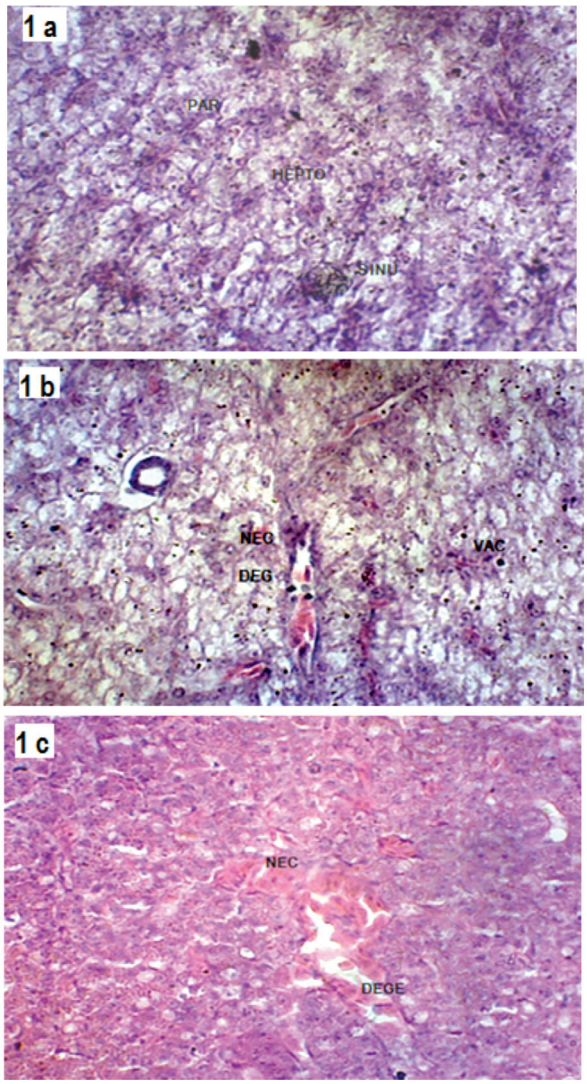

Fig. 1. Photomicrograph of a) Liver of control fish, b). Liver of treated fish after 15 days, c). Liver of treated fish after 30 days (all 40x magnification) PAR= Parenchymal cells, HEPTO= Hepatocytes, SINU= Sinusoids, VAC= Vacuolation, NEC=Necrosis, DEG=Degeneration

hepatic cells also observed. Sastry and Sharma (1981) exposed *Channa punctatus* (Bloch) to sub-lethal concntration (10.01 mg/L) of Endrin and observed hypertrophy of hepatic cells and vacuolation and necrosis of liver cells. Kabeer *et al.* (1978) reported cytoplasmic degenerative pyknotic in liver tissues, vacuolation in hepatic cells and ruptured blood vessels, hypertrophy in hepatic cells, necrosis etc. Several authors (Sulodia *et al.* 2014; Sepici-Dinçel *et al.* 2009; Sanjay *et al.* 2006; Anita and Tilak, 2003 and Maline, 1980) reported high frequency of hepatic hypertrophy, hepatic tumors and other liver disease of bottom dwelling fish due to pesticides. Similar histopathological changes in the liver were also reported by Gupta and Rajbanshi (1988); Narayan and Singh (1991); Das and Mukherjee (2000); Sanker and Jamel (2005).

Kidney: The kidney of control fish has intact nephrons and interstitial tissues. The microphotograph of kidney of *Channa punctatus* shows Bowman's capsule, in which proximal convoluted tubule and distal convoluted tubule are seen Fig. 2a.

The histopathological alterations that are seen

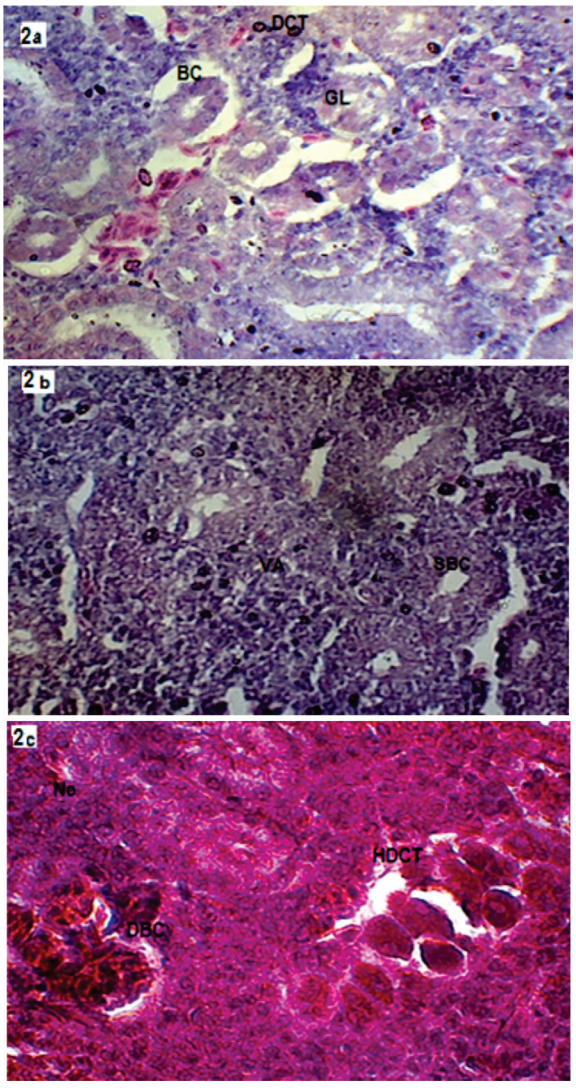

Fig. 2. Photomicrograph of a) Kidney of control fish, b) Kidney of treated fish after 15 days, c) Kidney of treated fish after 30 days, DCT=Distal convoluted tubule, GL= Glomerulus, BC=Bowman's capsule, SBC= Shrinkage of Bowman's capsule, VA= Vacuolation, HDCT= Hypertrophy of distal convoluted tubule, DBC= Dilation of Bowman's Capsule, Ne=Necrosis of interstitial cell

after exposure of Dithane M-45 are formation of vacuoles, necrosis of interstitial cell and dilation and shrinkage of Bowman's capsule. The 30 days exposure fish kidney showed the hypertrophy of distal convoluted tubules and swelling of renal tubules (Fig. 2 b,c).

Fishes when exposed to chemical pollutant causes several histopathological changes in the target organs including kidneys. Tubule degeneration, dilation of capillaries and changes in Bowman's capsule are some of the main alterations in fish exposed to pesticides (Anita and Tilak, 2003.) Wannee *et al*. (2002) reported kidney lesions consisting of dilation of Bowman's capsule space and accumulation of hyaline droplets in tubular epithelial cells of treated fish. Several other studies also showed similar changes in the histology of the kidney (Das and Mukherjee, 2000; Camargo and Martinez, 2007). The kidney is a critical which is regarded now as pollution indicator (Cengiz, 2006).

CONCLUSION

The present studies were done by using a sub lethal concentration of the pesticide for the evaluating toxic effects in fishes. The hazardous pesticides not only polluting the all spheres of environment but it cause huge damage to the biodiversity. The aquatic environments when polluted with toxic chemicals like Dithane M-45, inducing adverse effects on liver and kidney, toxic chemicals may accumulate in the fish tissue and then same may enter into the human body through food chain. Affected fish may produce less nutritive protein when the normal liver histology is disrupted. The liver necrosis of fish may be attributed to the destruction of the hepatoportal blood vessels which may results in the complete dissolution of the hepatocytes (Ram and Singh, 1988). Thus the results showed that the fungicide Dithane M-45 (Mancozeb) have potential to affect on fish fauna by causing medium to severe type of tissue damage and it can be said that fishes are able to uptake toxic pesticides dissolved in water through different biological processes.

REFERENCES

Anantham, G. and Kumaran, B. 2013. Effect of Mancozeb on the specific activities of Testicular phosphatase and protective role of vitamin C in Albino rats. *Bulletin of Environment, Pharmacology and Life Sciences* (BEPLS). 2 (7): 56-61.

Anita, S.T. and Tilak, K.S. 2003. Histopathological changes in the vital tissues of the fish *Cirrhinus mrigala* exposed to fenvalerate technical grade. *Pollut. Res.* 22 : 179-184.

Camargo, M.M.P. and Martinez, C.B. R. 2007. Histopathology of gills, kidney and liver of a Neotropical fish caged in an urbanstream. *Neotropical Ichthyology*. 5 : 327-336.

Cengiz, E. I. 2006. Gill and kidney histopathology in the freshwater fish *Cyprinus carpio* after acute exposure todeltamethrin. *Environ Toxicol Pharmacol*. 22 :200-204.

Chakraburthy, G. and Konar, S.K. 1974. Chronic effects pesticides on fish. *Proc Matl Acad Sci.* 446 : 241-246.

Das, B.K. and Mukherjee, S.C. 2000. A histopathological study of carp (*Labeo rohita*) exposed to hexachlorocyclohexane. *Veterinarsky Arhiv*. 70(4): 169-180.

Desai, A. K.; Joshi, U. M. and Ambadkar, P. M. 1984. Histological observations on the liver of *Tilapia mossambica* after exposure to monocrotophos, an organophosphorous insecticide. *Toxicol. Lett.* 21: 325 - 331.

Dutta, H.M. and Maxwell, L.B. 2003. Histological examination of sublethal effects of diazinon on ovary of bluegill. *Lepomis macrochirus*. *Environ. Pollut*. 121 : 95-102.

EIFAC. 1983. Working party on toxic testing procedures, revised report on fish toxicology procedures. *EIFAC Technical paper*. 24: 1-37

Finney, P.J. 1971. Probit analysis 3rd edition. Cambridge University Press,Cambridge, London (UK).

Gupta, A.K. and Rajbanshi, V.K. 1988. Acute toxicity of cadmium to *Channa punctatus*. *Acta. Hydrochim. Hydrobiol*. 16 (5): 525-535.

Hayes, W.J. and Laws, E.R. 1991. Handbook of Pesticide Toxicology. Vol. 3. Classes of Pesticides. New York: Academic Press Inc. pp. 1451 .

Jayantha Rao, M. K. and Rama Murthy, K. 1985. Histopathological and histochemical changes under phosphomidon intoxication in liver of freshwater fish *Tilapia mossambica*. *Proc. Bull. Environ. Sci.* 3 : 20-23.

Kabeer, A.I., Begum, R., Siviah, S. and Ramana Rao, K.V. 1978. Effect of malathion on free amino acids, total protein, glycogen and some enzymes of pelecyped, *Lamellidenns marginalis*. *Proc. Ind. Acad. Sci*. 87: 377-380.

Kumari, A. S. and Kumar, N. S. R. 1997. Effects of water pollution on histology of intestine of two fresh water fishes from Hussainsagar Lake (A. P.), *Indian J. Environ. Toxicol.* 7 : 68-70.

Mason, C.F. 1991. *Biology of Freshwater Pollution.* 2nd Edition, Longman Scientific and Technical U.K. pp351

Maline, D.C. 1980. Chemical contaminants and biological abnormalities in Central and Southern puget sound.

NOAA Technical memorandum OMPA/ U.S. Department of Commerce, National Oceanic and Atmospheric Administration, Boulder Colo. pp. 295

Mathur, D.S., Agarwal, H.D. and Rana, P.D. 1981. Histopathological changes in the liver and intestine of *Rana cyanophyticlis* (Sehne) induced by aldrin. *J. Environ. Biol.* 2 : 105-107.

Muhammed, A. and Telat, Y. 2003. Alterations in hematological parameters of Rainbow Trout (*Oncorhynchus mykiss*) exposed to Mancozeb. *Turk J Vet Anim Sci*. 27 : 1213-1217.

Narayan, A.S. and Singh, B.B. 1991. Histopathological lesions in *Heteropneustes fossilis* subject to acute thiodon toxicity. *Acta Hydrochem. Hydrobiol.* 19: 235-243.

Nwani, C.D., Lakra, W.S., Nagpure, N.S., Kumar, R., Kushwaha, B. and Srivastava, S.K. 2010. Mutagenic and genotoxic effects of Carbosulphan in freshwater fish *Channa punctatus* (Bloch) using micronucleus assay and alkaline single-cell gel electrophoresis. *Food Chem Toxicol.* 48 : 202-208.

Pandey, A.C. 1988. Impact of Endosulfan (ThiodanEC 35) on behavior and dynamics of oocyte development in the teleostean fish *Colisa fasciatus*. *Ecotoxic. Environ. Safety*. 15 : 221-225.

Ram, R. N. and Singh, S. K. 1988. Carbofuran - induced histopathological and biochemical changes in liver of the teleost fish; *Channa punctatus* (Bloch). *Ecotoxicol Environ. Safety*. 75 (3) : 194-201.

Ramesh, A. and Maheswari, S.T. 2004. Dissipation of alachlor in cotton plant, soil and water and its bioaccumulation in fish. *Chemosphere*. 54: 647-652.

Sanjay, P., Ravindra, K., Shilpi, S., Nagpure, N.S., Satish, K., Srivastava, S. and Verma, M.S. 2006. Acute toxicity of chromium exposed *Channa marulius*. *Indian J Environ*. 38 : 118-121.

Sanker, S.A. and Jamel, S.M. 2005. Fenvalerate induced histopathological and histochemical changes in the liver of the cat fish *Clarias batrachus*. *J. Appl. Sci. Res*. 1 (3): 263-267.

Sastry, K. V. and Sharma, K. 1981. Diazinon-induced histopathological and haematological alterations in a freshwater teleost, *Ophiocephalus punctatus. Ecotoxicol. Environ. Safety*. 5 : 329-340.

Sepici-Dinçel, A., Benli, A. Ç. K., Selvi, M., Sarýkaya, R., Þahin, D., Ozkul, I. A. and Erkoç, F. 2009. Sublethal cyfluthrin toxicity to carp (*Cyprinus carpio* L.) fingerlings: biochemical, hematological, histopathological alterations. *Ecotoxicology and Environmental Safety*. 72(5) : 1433-1439.

Shahi, J. and Singh, A. 2014. Genotoxic and haematological effect of commonly used fungicide on fish *Clarias batrachus*. *J Biol Earth Sci.* 4 : B137-B143.

Sulodia, S., Singh, S. and Sharma, H. N. 2014. Histopathological changes in liver of C*hanna punctatus* (bloch) under stress of folidol. *Ind J Biol Stud Res*. 3(2) : 109-119.

Sun, F., Lin, F.Y., Wong, S.S. and Li, G.C. 2000. Determination of Organochlorine and Nitrogen-containing pesticide residues fish with different fat content. *J. Food & Drug Analysis*. 8 : 103-112.

Tilak, K.S., Veeraiah, K. and Koteswara Rao, D. 2005. Histopathological changes in the gill, liver, brain and kidney of the Indian major carp *Cirrhinus mrigala* (Hamilton) exposed to chloropyrifos. *Pollut. Res*. 24: 101-111.

Wannee, J. K., Upatham, E S., Kruatrachue, M., Sahaphong, S., Vichasri-Grams, S. and Pokethitiyook, P. 2002. Histopathological effects of round up, a glyphosate herbicide, on Nile tilapia *Oreochromis niloticus*. *Science Asia*. 28 : 121-127.