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Research Article

Histopathological changes induced by dimethoate in the liver of freshwater fish *Puntius ticto* (Ham)

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Abstract

The present investigation deals with the histopathological changes induced by dimethoate on the liver of *Puntius ticto* (Ham). *Puntius ticto* a freshwater fish exposed to lethal (5.012ppm) and two sub-lethal (2.506 & 1.253 ppm) concentration of dimethoate for acute (four days) and chronic (sixty days) exposure. After lethal exposure liver showed significant changes. Marked swelling and compact arrangement of hepatocytes were observed. Vacuoles were seen in the cytoplasm and nuclei continue to be pyknotic and eccentric. There were cloudy swellings of the cells with large vacuoles, degenerated sinusoids, disorganized hepatic cords and loss in shape of hepatocytes was observed. Long-term exposure also showed swelling of hepatocytes, vacuolation in the hepatocytes, disorganized hepatic cords and loss of shape of hepatocytes i.e., cell wall rupture. Lining of bile duct was also observed ruptured, cell necrosis and focal fibrosis was observed in both the sub-lethal exposures. Vacuoles were also seen in the cytoplasm of hepatocytes and nuclei continued to be pyknotic and eccentric. The granulotoma condition was observed in all concentrations during short and long-term exposure. The above changes showed that the severity of damage is dependent upon the dose of concentration.

INTRODUCTION

India is primarily an agro-based country where more than 60-70% of its population depends on agriculture. However, 30% of its agricultural produce is lost owing to pest infestation. Due to the absence of better alternative deployment of pesticides become inevitable despite their known hazardous effects. In India utilization of pesticides is about 3% of total world consumption and is increasing at the rate of 2-5% per annum (Bhadbhade *et al.*, 2002). Extensively used pesticides in agriculture, forestry, public health and veterinary practices are run off from fields into the ponds, lakes and rivers and causing serious concern about water pollution. Pesticides and related chemicals destroy and eradicate the economically important species either indirectly through breaking the biological chains or directly produces toxic stress and chemical changes. Predominantly, as a result of extensive application of pesticide, large scale mortalities of fish occurred widely (Srivastava and Srivastava, 1994). Ten million of fish are killed every year by a wide variety of different pollutants from many sources like municipal, agricultural and industrial. From industrial and agricultural operations, these compounds find their way in to the natural water resources and affect the aquatic organism (Tilak *et al.*, 2007).

The freshwater fishes constitute one of the major sources of nutritious food for humans. Fish

are largely being used for assessment of the quality of aquatic environment and as such can serve as bioindicators of environment pollution. Among the aquatic species, the fish are the major targets of toxicants like pesticides. Hence pollutants such as pesticides may significantly damage certain physiological and biochemical processes when they enter into the organs of fishes (John, 2007; Banaee *et al.,* 2011). Due to accumulation of these pesticides in tissues produces many physiological, histological and biochemical changes in the fishes and freshwater fauna by influencing the activities of several enzymes and metabolites (Nagarathnamma & Ramamurthi, 1982). Fish health may thus reflect, and give a good indication of the health status of a specific aquatic ecosystem. Histological analysis appears to be a very sensitive parameter and is crucial in determining cellular changes that may occur in target organs, such as the gills, liver and kidney (Dutta, 1996). Therefore, histological investigation may be cost effective diagnostic tool for infectious and non-infectious fish diseases and can therefore be used to assess the health of fish populations by analyzing selected target organs (Van Dyk and Pieterse, 2008). Dimethoate is an organophosphorus insecticide widely used against vegetable and fruit sucking aphids, mites and saw flies. Exposure to chemical pollutants may cause many molecular, biochemical changes in the fish which precede cellular and systemic dysfunctions. So that, if appropriate parameters are monitored, early warning signs of distress may be detected (Palmes, 1976). However there has been little information regarding the adverse effect of dimethoate on the liver of fishes. Keeping this in mind present study was undertaken to assess the toxicological manifestation of a dimethoate on the liver of freshwater fish, *Puntius ticto.*

MATERIAL AND METHODS

The freshwater fish *Puntius ticto* were collected from the freshwater sources around Aurangabad city. They were acclimatized in aged, dechlorinated and well aerated water for two weeks in the laboratory. During acclimatization they were fed on alternate days with pieces of live earthworms. The LC_{50} values are determined by following the guidelines given by committee of toxicity tests with aquatic organism (Annon, 1975) and Probit Analysis Method (Finney, 1971). The 20 healthy and acclimated fishes showing normal activity were exposed to lethal concentration (5.012ppm) for 96h (Acute Study) and sublethal concentrations (2.506ppm and 1.253ppm) for 60 days (Chronic Study). Simultaneously a control group of healthy fishes were maintained under identical conditions. After commencement of exposure period fishes were killed by decapitation and liver is removed and fixed in Bouins fluid for 24h and processed according to standard procedure of routine microtechnique. For staining double stain method was followed by using Hematoxylin and Eosin and mounting was done in DPX.

RESULTS AND DISCUSSION

Histology of Liver:

The single lobe of a normal liver section shows that it is formed of tiny irregular lobules separated by thin connective tissue partitions. The lobules are filled with polygonal cells called as parenchymal cells or hepatocytes. Polygonal cells having distinct central nuclei with nucleoli. The hepatocytes are arranged in cords, filled with clear cytoplasm. The blood spaces are lined by connective tissue (Fig A)

Histopathology of Liver:

Fish exposed to lethal concentration of dimethoate showed marked changes in the histological architecture of liver. Marked swelling and compact arrangement of hepatocytes were observed. Vacuoles were seen in the cytoplasm and nuclei continue to be pyknotic and eccentric. There were cloudy swellings of the cells with large vacuoles, degenerated sinusoids, disorganized hepatic cords and loss in shape of hepatocytes was observed. The inter-cellular space became wider due to connective tissue damage. Shrinkage and dilation of blood vessels was also observed. Haemorrhage, widening of blood sinusoids and rupture in the lining of bile duct was observed (Fig. B).

Long-term exposure also showed swelling of hepatocytes, vacuolation in the hepatocytes, disorganized hepatic cords and loss of shape of hepatocytes i.e., cell wall rupture at both the sub-lethal concentrations. Lining of bile duct was also observed ruptured, cell necrosis and focal fibrosis was observed in both the sub-lethal exposures. Vacuoles were also seen in the cytoplasm of hepatocytes and nuclei continued to be pyknotic and eccentric. These changes were prominent in higher concentration than at lower concentration. The granulotoma condition was observed in all concentrations during short and long-term exposure. The above changes showed that the severity of damage is dependent upon the dose of concentration

and exposure time (Fig C, D, E and F). Liver plays a key role in maintaining the internal homeostasis in vertebrates.Having a rather dynamic structure, it is a good study model since it regulates many, metabolic and physiological processes (Signer 1998). Liver also is the main organ of detoxification (Dutta *et al.,* 1993), it regulates metabolism, transforms, excrete xenobiotics and helps in detoxification, that suffers serious morphological alterations in fish exposed to pesticides (Rodrigues and Fanta 1998). Alterations in the liver may be useful as a marker that gives prior indication of physiological alterations on exposure to environmental stressors such as pesticides. Tissue injuries and damage caused by pesticide resulted to reduced survival, growth, fitness and the low reproductive success or increase of susceptibility to pathological agents (Lakshmaiah, 2016)

Bhattacharya and Mukherjee (1975) studied effect of endrin on hepatopancreas of *Clarias batarachus* and observed degenerative changes as liver cord disarray, vacuolation, necrosis, rupture, vacuolation of the hepatic cells with binucleated and enlarged nucleus. They also reported that histopathological effects of endrin were proportionally greater in fishes exposed to higher concentration of endrin. Robert and Summerfelt (1975) studied cadmium induced histopathological changes in gold fish and observed granulotoma formation in the liver. Sastry and Sharma (1979) studied endrin induced changes in liver histology of *Channa punctatus* and observed swollen hepatic cells, liver cord disarray, necrosis vacuolation in cytoplasm, increase in size of nucleus, ruptured cell membrane and widened inter-cellular spaces due to connective tissue damage. Similar results were observed in the *Puntius ticto* during present investigation.

Mandal and Kulshrestha (1980) studied histopathological changes induced by sumithion in *Clarias batarachus* and observed severe necrosis on 90th day than the 45th day. Loss of polygonal shape of hepatic cells, vacuolation in cells, rupture and disintegration of cellular wall which leads to formation of multinucleate giant cells. Hanumante *et al.,* (1981) reported disarray of hepatic cords, clumped hepatocytes and prominent increase in blood sinusoids in *Channa gachua* treated with mercuric chloride. Kumar and Pant (1981) showed vacuolation within and outside the hepatocytes and severe necrotic changes in the liver of *Puntius conchonius* on copper and zinc treatment. Kamble (1983) studied histopathological changes in the liver of *L. thermalis* exposed to sumithion and BHC. Exposure to BHC showed vacuolated hepatic cells, pyknotic nuclei, coagulation of hepatocytes and shrinkage of parenchymal cells; while sumithion toxicity resulted in vacuolation in hepatic cells, swelling of parenchymal cells with pyknotic nuclei, liver cord distortion and necrosis in parenchyma with degenerative lesions. Khalid *et al.,* (1986) studied histopathological changes in the liver of *B. ticto* and *R. daniconius* exposed to suquin and recorded hyperplasia, rupture of hepatocytes, pyknotic nuclei, denucleated hepatocytes and aggregation of nuclei and vacuolation. Ramlingam (1988) studied effect of DDT, malathion and mercury on liver morphology of *Sarotherodon mossambicus* and reported changes like necrosis, fatty degeneration and red cell occlusion in portal vessel, engorged blood vessels congestion, vacuolar degeneration of hepatocytes, necrosis of pancreatic cells and fatty changes in peripancreatic hepatocytes.

Radhaiah and Rao (1992) have exposed *T. mossambica* to sub-lethal concentration of fenvalerate for 10 and 20 days and noted histopathological lesions such as vacuolated hepatocytes, cell necrosis, movement of nuclei to the cell periphery, pyknotic nuclei and cytoplasmic degenerations in the liver tissue. Dutta *et al.,* (1993) observed compactly arranged hepatic cells with prominent nuclei and nucleolus, shrinkage of hepatic cells, degeneration of cell membrane; vacuolation in the cytoplasm, nuclei became pyknotic and eccentric after 48 hrs. exposures to malathion in *Heteropneustes fossilis*. After 96 hrs. exposure they observed vacuolization, pyknotic and eccentric nuclei, displacement of nuclei, rupture of membrane, binucleate or multinucleate cells at several areas and some cells became necrotic and complete extrusion of nuclei. Moza *et al.,* (1993) studied effect of cadmium on liver of *Carassius auratus* and observed necrotic cells, fibrosis, granulotoma, dilated sinusoids with full RBC on 15th day and on 40th day they observed pathological lesions in liver parenchyma, cell wall lysis, swollen hepatocytes, granulated cytoplasm, vacuolation within cytoplasm and hepatocytes lost polygonal shape. Dwivedi and Sarin (1996) studied histopathological changes in the liver of the cat fish *Heteropneustes fossilis* induced by triaromatic hydrocarbon. They observed severe necrosis, pyknosis and disintegration of hepatocytes cells on the 30th day. The complete disintegration of

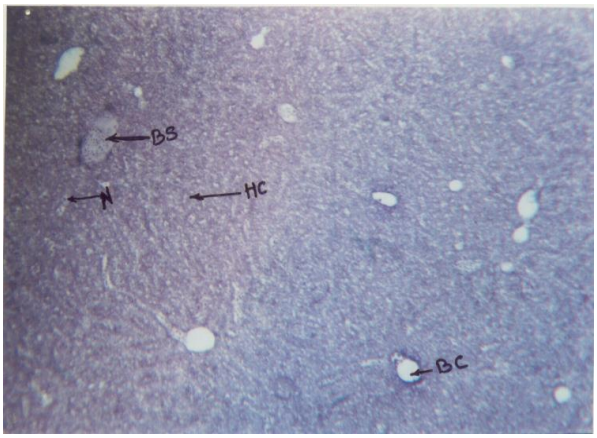

Fig A) T.S. of liver of *Puntus ticto* (Control), Haematoxylin/Eosin 100X
BC-Bile canaliculi, **BS**-Blood sinusoids
HC-Hepatocytes
N-Nucleus

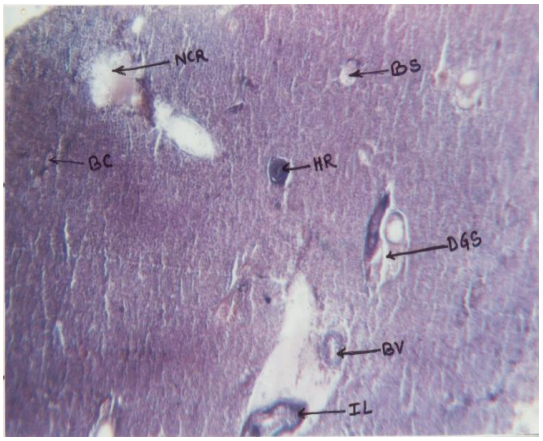

Fig B) T.S. of liver of *Puntius ticto* after 5.012 ppm exposure to dimethoate,Haematoxylin/Eosin 100X
BC-Bile canaliculi, BS-Blood sinusoids
BV-Blood vessel
DGS-Degenerated blood sinusoids
HR-Hemorrhage, IL-Islets of Langerhans
NCR-Necrosis

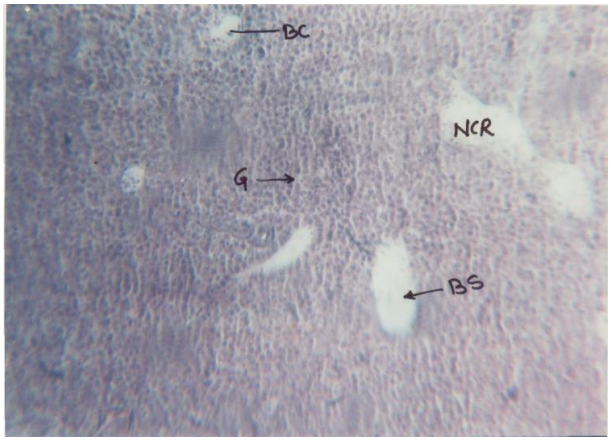

Fig.C) T.S. of liver of *Puntius ticto* after 2.506 ppm exposure dimethoate Haematoxylin/Eosin 200X
BC-Bile canaliculi, BS-Blood sinusoids
G-Granulotoma, NCR-Necrosis

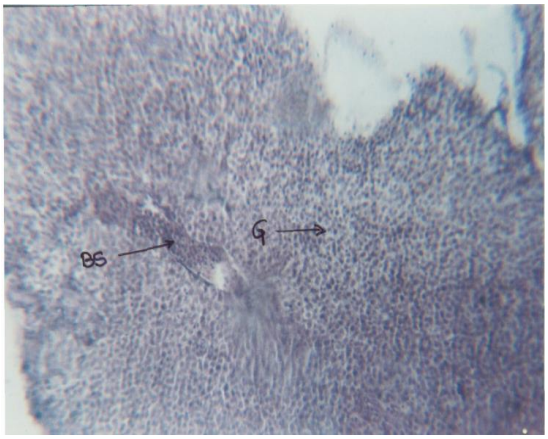

Fig. D) T.S. of liver of *Puntius ticto* after 2.506 to dimethoate, Haematoxylin/Eosin 100X
BS-Blood sinusoids
G-Granulotoma

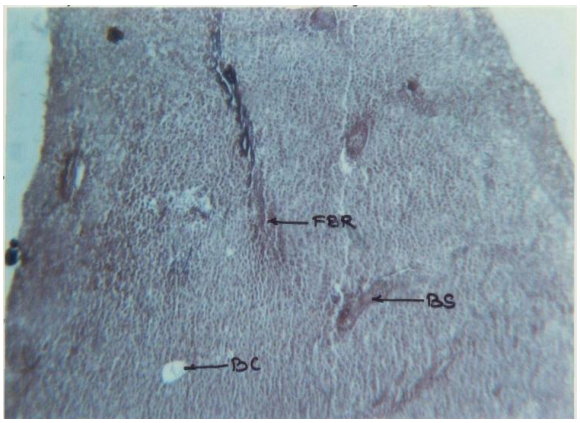

Fig.E) T.S. of liver of *Puntius ticto* after 1.253 ppm exposure to dimethoate, Haematoxylin/Eosin 100X
BC-Bile canalicule BS-Blood sinusoids

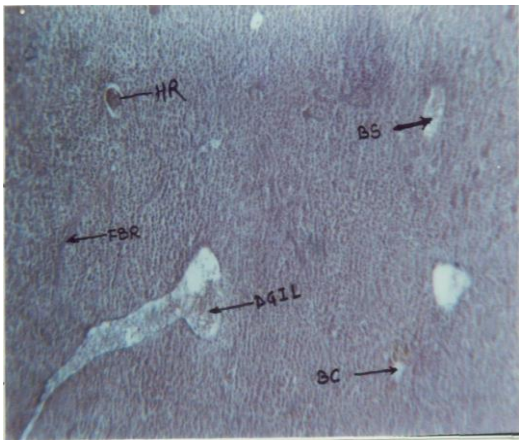

Fig.F) T.S. of liver of *Puntius ticto* after 1.253 ppm exposure to dimethoate, Haematoxylin/Eosin 100X BC-Bile canaliculi BS-Blood sinusoids

hepatocyte leading to tumor formation is indicative of carcinogenic degeneration, oedema formation, hypertrophy and atrophy were observed. Banerjee and Bhattacharya (1997) reported the vacuolation of the hepatocytes, pyknosis in many necrotic cells, necrosis of the exocrine pancreatic tissue and disintegration of sinusoids in the hepatopancreas of *Channa punctatus* exposed to elsan, mercury and ammonia for 90 days. Das and Mukherjee (2000) observed marked swelling of hepatocytes, diffuse necrosis, disrupted architecture of liver tissue, distended sinusoids, damaged central veins and degeneration of endothelial lining cells in *Labeo rohita* treated with hexachlorocyclohexane. Cengiz *et al.,* (2001) studied effects of thiodon on the liver of *Gambusia affinis* and observed degeneration, hypertrophy, sinusoid enlargement, hemorrhage, pycnosis, displacement of nuclei, vacuolization of cell cytoplasm, infiltration of mononuclear lymphocyte and congestion in liver. Similar results were observed during present investigation. Sakr *et al.,* (2001) studied the effect of organophosphorus insecticide (Hostathion) on the liver of the catfish *Clarias gariepinus* and found liver cord disarray, cytoplasmic vacuolization of the hepatocytes, damage of blood sinusoid, blood vessel congestion and inflammatory leucocytic infiltrations. Sakr and Jamal (2005) observed cytoplasmic vacuolization of hepatic cells, inflammatory leucocytic infiltrations, congestion of blood vessel, necrosis and fatty infiltrations in the liver of catfish *Clarias gariepinus* exposed to fenvalerate. Ayoola and Ajani (2008) observed severe necrosis, hemorrhage and vacuolization in the liver of cypermethrin treated fish Nile tilapia *Oreochromis niloticus*. Velmurugan *et al.,* (2009) observed cloudy swelling of hepatocytes, congestion, degeneration, karyolysis, karyohexis, dilation of sinusoids and nuclear hypertrophy in the liver of freshwater fish *Cirrhinus mrigala* exposed to dichlorvos. Lawrence and Ogbomida (2010) studied histopathological effects of gammalin 20 on the liver of African catfish *Clarias gariepinus* and observed mild necrosis, swelling of blood vessels, pyknosis and vacuolization. Radhakrishnan and Hemalatha (2010) observed large number of hepatocytes with pyknotic nuclei, dilated and congested hepatic blood vessels, inflammatory leucocytic infiltration, hepatocytes with marked cytoplasmic vacuolization, distended sinusoids, marked swelling and degeneration in the endothelial lining cells in the liver of *Channa striatus* exposed to cadmium chloride. Tripathi *et al.,* (2011) studied lindane intoxication in the freshwater fish *Catla catla* and reported swollen and ruptured parenchymal cells, loss of cord structure, vacuoles filled with cellular debris, focal necrosis and significant increase in Kupffer cells. Hasina and Mithra (2014) studied histopathological changes in the liver of *Heteropneustes fossilis* under chlorpyrifos 20EC intoxication and reported congestion of central vein, degeneration of hepatocytes, cytoplasmic vacuolization, hepatocytes with pyknotic nuclei, thrombosis in hepatoportal blood vessel, hemorrhage around central vein and necrosis. Lakshmaiah (2016) studied effect of phorate on the liver of *Cyprinus carpio* and observed severe degree of atrophy of liver cords, degeneration of hepatocytes, cloudy swelling of hepatocytes, nuclear hypertrophy and nuclear degeneration along with focal necrosis. Srinivasarao *et al.,* (2018) observed prominent degeneration and necrosis, degeneration of hepatocytes, prominent atrophy, vacuolated cytoplasm, ruptured blood vessels and disposition of hepatic cords in the liver *Ctenopharyngodon idella* under deltamethrin 11% EC exposure.

CONCLUSION: Significant Histopathological alterations were observed in the liver of *Puntius ticto* under dimethoate toxicity. swelling of hepatocytes, vacuolation in the hepatocytes, disorganized hepatic cords and loss of shape of hepatocytes, cell necrosis and focal fibrosis, Vacuolated hepatocyte with pyknotic and eccentric nuclei and granulotoma. These histopathological changes in the liver are not only dependent on the concentration of the pesticide but also on the length of the fish exposure period.

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