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EFFECT OF 2,4- DICHLOROPHENOXY ACETIC ACID (2,4-D) ON LIVER OF A FRESH WATER CATFISH, *HETEROPNUESTES FOSSILIS* (BLOCH)

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ABSTRACT – Liver of *Heteropneustes fossilis* exposed to sub-lethal concentration of 2,4-D showed a number of degenerative changes in hepatic cells. The extent of liver damage observed in the present investigation indicates that herbicide exposure must be causing impairment in the liver function of detoxification of the poisons with nutritional deficiency disease in fishes. The main site of metabolic activation of toxicant is liver, so the hepatic tissue damage produced by the toxicant will undoubtedly impair the metabolic status of this vital organ. This in turn will result not only in lowered synthesis of reserve food materials namely, glycogen, fat and protein, but also in the breakdown of these substances which are vital for maintaining most of the metabolic activities of cell.

Key words : 2,4-D, *Heteropnuestes fossilis,* liver.

INTRODUCTION

Heteropneustes fossilis (Bloch.) is live catfish and inhabits all kinds of streams, irrigational channels marshes, swamps and even sewage-fed tanks. They always live in shoals showing wriggling and sepentine movements at the bottom under big boulders and amongs marshy beds. Occasionally they come close to the surface forming congregations particularly in the ponds or puddles covered with floating weeds or algal blooms, for gulping atmospheric oxygen. The frequency of the visits to the surface varies at different times of the day. It is very much dreaded for its poisonous pectoral spine and a poisonous gland at the base of spine. They are predacious and carnivorous. They belong to the family Heteropneustidae and order Siluriformes.

2,4-D is 2,4-Dichlorophenoxy acetic acid and is used in herbicides. 2,4-D is a chlorinated phenoxy compound. The molecular formula of 2,4-D is $C_8H_6Cl_2O_3$. It functions as a systemic herbicide and is used to control many types of broadleaf weeds. This compound is used in cultivated agriculture and in pasture and rangeland applications, forest management, home and garden situations and for the control of aquatic vegetation. It is the most widely used herbicide in the world. The main site of metabolic activation of toxicant is liver (Siddiqui *et al*, 1993; Ahmed, 1998; Sinha *et al*, 1988; Begum *et al*, 1995; Kaundinya *et al*, 1979; Philip *et al*, 1995, Singh *et al*, 1982a, 1982b; Singh *et al*, 1997a, 1997b; Srivastava *et al*, 1982), so the hepatic tissue damage produced by the toxicant will undoubtedly impair the metabolic status of this vital organ. This in turn will result not only in lowered synthesis of reserve food materials namely, glycogen, fat and protein, but also in the breakdown of these substances which are vital for maintaining most of the metabolic activities of cell.

Widespread use of various pesticides, including 2,4-D and their consequent leaching to water, causes the aquatic ecosystem to get polluted and the populations of organisms, including fishes, suffer on account of the toxic nature of Herbicides. The ill-effect of 2,4-D not only harms the fishes but also all fish eating animals including human beings. Therefore, to avoid the pollution of aquatic ecosystems by 2,4-D, a sincere attempts is required for making their use judicious. Regular monitoring to maintain the level of pesticides within safe limits is evidently the most important (Basak and Konar, 1977a, 1977b).

The present investigation is an attempt to evaluate the toxicological effects of 2,4-D on liver of *Heteropnuestes fossilis*.

MATERIALS AND METHODS

Live specimens of *Heteropneustes fossilis* (approx. length 15±3 cm and wt. 50 ± 10g) were collected from local ponds and river Gomati at Jaunpur city (U.P.) and were acclimated to optimum lab conditions for 15 days. Fish during acclimatization were fed with minced goat liver on alternate days. Fishes were exposed to sublethal concentration (0.6 mg/litre) of 2,4-D. Fishes were cold anaesthesized following Mittal and Whiter (1978) and liver from both sides of fishes were excised, rinsed in saline water and were fixed in 10% neutral formation and Bouin's aqueous at 15 days, 30 days, 45 days, 60 days and 90 days of 2,4-D treatment. Standard methods of

dehydration, clearing and embedding were used. Paraffin sections were cut at 5 µm thick and stained with Ehrlich's Haemeatoxylin/Eosin (H/E) to study structure of liver.

OBSERVATION

Control :

Histological architecture of the liver of control *Heteropneustes fossilis* reveals polygonal hepatic cells with homogeneous cytoplasm with distinct central nucleus having round nucleolus (Fig.1). The pancreatic cells of hepatopancreas are arranged around a branch of portal vein .

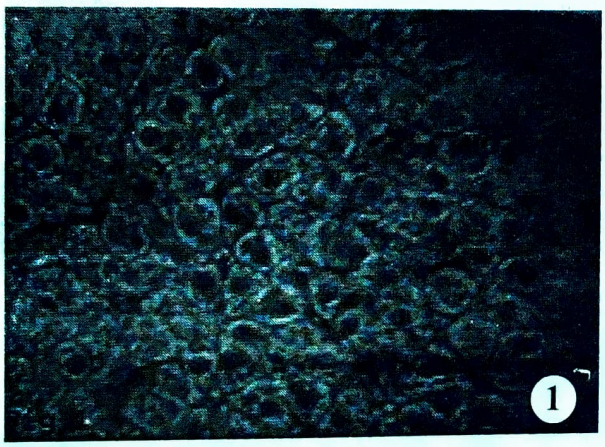
2,4-D Exposure:

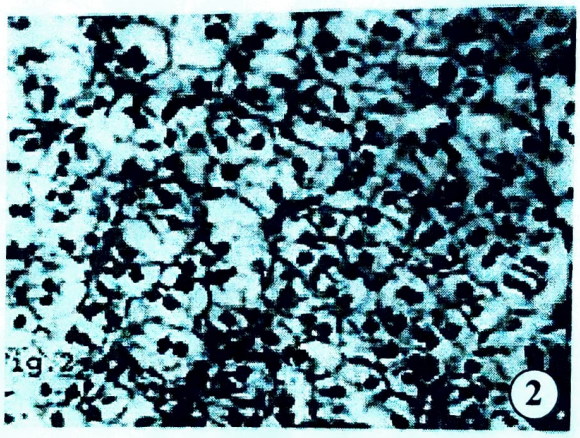
Hepatic damage is well demonstrated histologically as the normal histoarchitecture of the hepatocytes observed in liver of control fish is disrupted on herbicide exposure.

After 15 days exposure, the liver showed swollen hepatocytes with nuclear hypertrophy (Fig.2). After 30 days treatment vacuolization (V) in cytoplasm of hepatocytes is well marked (Fig.3) After 45 days exposures, necrosis is observed in most of the hepatocytes. These cells show pyknotic nuclei and disintegrated cell boundary. After 60 days treatment, most of the hepatocytes get degenerated as indicated by heavy vacuolization (V), rupture of their cell membranes and pyknosis of nuclei. Only a few cells have nuclei, though they also lose their membranous covering (Fig.4). After 90 days exposure, blood is observed, oozing among the disintegrated hepatocytes . The exocrine hepatopancreatic cells reveal various degree of degeneration indicated by hypertrophy and nuclear

DISCUSSION

Liver of *Heteropneustes fossilis* exposed to 2,4-D





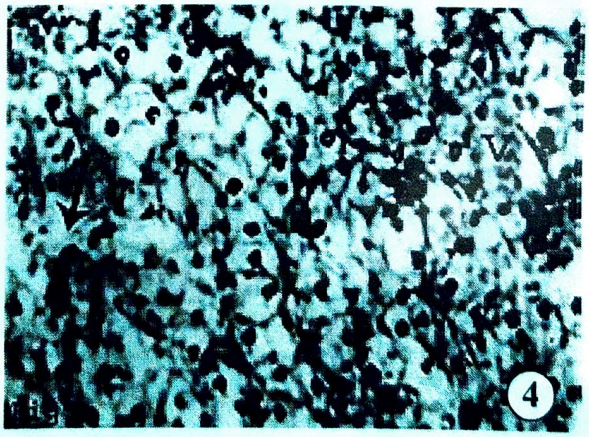


Fig 1 : Photomicrograph of the cross section of liver of control *Heteropneustes fossilis* having polygonal hepatic cells (HC) with distinct nucleus having nucleolus. (HE) X 400.

Fig 2 : Photomicrograph of the cross section of the liver of control *Heteropneustes fossilis* exposed to 2, 4-D at 15 days showing swollen hepatocytes with hypertrophied nucleus having distinct nucleolus (HE) X400.

Fig 3 : Photomicrograph of the cross section of the liver of *Heteropneustes fossilis* after 30 days exposure to 2, 4-D, showing vacuolization (V) in hepatocytes. (HE) X 400.

Fig 4 : Showing heavy disintegration in hepatocytes of *Heteropneustes fossilis* in exposed to 2, 4-D at 60 days. Note vacuolization (v) and pycnosis (arrow). (HE) X 400.

showed a number of degenerative changes in hepatic cells. This is in agreement with the earlier reports which describe toxicants– induced hepatic cytopathology from other fish species. Srivastava *et al* (1998) have observed histopathological changes in the liver of fish *Heteronpnuestes fossilis* which induced by Malachite green. Heptachlor induced degenerative changes in liver of *Lepomis macrochirus* have been reported by Andrews *et al* (1966). Amminikutty and Rege (1977) have reported focal necrosis in hepatocytes of *Gymnocorymbus ternitzi* after agallol '3' treatment. Hypertrophy and pycnotic nuclei were observed in *Channa punctatus* when this fish was exposed to mercuric chloride (Sastry and Gupta, 1978) and urea (Srivastava and Srivastava, 1979). Granular dystrophy and cyloplasmic vacuolisation in liver of Asian catfish exposed to dimethoate has been noticed by Anees (1976). Gill *et al* (1988) reported hypertrophy, vacuolisation and necrosis of hepatocyts in carbaryl and dimethoate treated *Puntius conchonius*. Srivastava et.al., (1990) have reported similar finding in the liver of *H.fossilis* exposed to chlorpyrifos. In case of *H.fossilis*, previous studies have shown the occurence of vacuolated hepatocytes with general loss of staining response on exposure to sewage (Narain *et al,* 1990) and shrunken and vacuolated cytoplasm and pycnotic nuclei after exposure to thiodon (Narain and Singh, 1991). Shukla (1995) reported degenerative changes in liver following pesticide poisoning in fish *C. fasciatus*. Okihiro and Hinton (1999) have reported hepatic neoplasia in medaka, *Oryzias latipes*, following aqueous exposure to dehylintrosomine (DEN). Poleksic and Karan (1999) investigated the subacute toxicity of the herbicide trifluralin on fish liver and found its intensity to decrease in relation to growth rate. The extent of liver damage observed in the present investigation indicates that herbicide exposure must be causing impairment in the liver function of detoxification of the poisons with nutritional deficiency disease in fishes (Eller, 1971; Wedemeyer *et al,* 1976; Cowey and Roberts, 1978; Roberts, 1978; Owen and Rosso, 1981). But, as also concluded earlier (Narain and Singh, 1991), a nutritional deficiency developing within only 96 hrs. period is not very likely. So degeneration of cytoplasmic substance under a direct action of herbicide would be the likely cause of the generally weakened cytoplasmic staining observed presently in the liver cells of stressed *H.fossilis*.

The main site of metabolic activation of toxicant is liver (Siddiqui *et al*, 1993; Ahmed, 1998; Sinha *et al,* 1988; Begum *et al,* 1995, Kaundinya *et al*, 1979, Philip *et al*, 1995, Singh *et al*, 1982a, 1982b, Singh *et al,* 1997a, 1997b, Srivastava *et al*, 1982), so the hepatic tissue damage produced by the toxicant will undoubtedly impair the metabolic status of this vital organ. This in turn will result not only in lowered synthesis of reserve food materials namely, glycogen, fat and protein, but also in the breakdown of these substances which are vital for maintaining most of the metabolic activities of cell. Robert (1978) is of the opinion that the changes in the liver of fish are due to a failure of the sodium pump across the cell membrane.

The liver of fish does not show the diversity of pathology seen in higher animals. However, its susceptibility to a number of toxic and the consequential metabolic disturbances cannot be overemphazied (Roberts, 1980; Olojo *et al*, 2005). The high proportion of fibrotic tissue within the lobules and peribiliary connective tissue of the treated specimens indicate hepatic cirrhosis. It is thus believed that the most dramatic cirrhosis found in fish is the peribilliary cirrhosis of the hepato-renal syndrome associated with dietary toxicity (Anderson *et al,* 1976). The most frequent of the degeneration was hepatocytes enlargement with large vacuoles and sinusoid conjection, pyknosis and karyolysis observed in cases of severe intoxication with pollutants (Jiraungkoorskal *et al,* 2003).

During the exposure of 2,4-D; the shrinkage of the hepatic cells can result in cirrhosis the contracting of the blood vessels thereby greatly impeding the portal flow through the liver. The functions of the liver such as the conversion of glucose to glycogen for storage, regulation of lipids and deamination of amino acids are impaired.

The blockage of the sinusoids makes the blood flow from the hepatic artery and veins into the central vein rather difficult. The sinusoids widened to make up the right volume of blood in the central vein. The function of the canaliculi that forms the bile duct is hampered and as such, bile secreted from gall bladder cannot adequately get into them.

In conclusion, the exposure of *H.fossilis* to 2,4-D caused histopathological alterations in liver tissue. Because its concentration in streams is often at sub-lethal levels, the histopathological effect are gradual. This may not result in fish kill immediately but definitely represents a health hazard to human consumers.

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