Histopathological Alterations in the Liver, Gills and Kidneys of Fish *Labeo Rohita* After the Exposure of Endosulfan and Lindane

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Abstract— Pesticides have become an important tool of modern agriculture to protect standing crops, stored grains and human belongings from pests and also help in preventing diseases. As a whole or in residual form, these pesticides make their entry into the aquatic ecosystem and pose a serious threat to the aquatic organisms in general and fishes in particular. To evaluate the effects of selected pesticides (Endosulfan and Lindane), histological studies on liver gills and kidney tissues of fish, *Labeo rohita*, were investigated. On exposure to the above said chemicals, liver recorded congestion, hemorrhages, micro to macro-vacuolar degeneration of hepatocytes, mononuclear cell infiltration, bile duct hyperplasia, necrosis and periductular fibrosis. While coagulative necrosis of hepatocytes was recorded during Endosulfan exposure only. Gills recorded multifocal necrosis of lamellae, congestion and hemorrhages of blood capillaries and fusion & atrophy of secondary lamellae after exposure to Endosulfan and Lindane. Kidney showed mild congestion and hemorrhages of blood vessels with mild tubular degeneration, diffused swelling and degeneration of proximal and distal convoluted tubules, narrowing of renal tubules and loss of hemopoetic tissue. The physic-chemical parameters of water remained in the optimum limits during the experimental period.

Keywords— Endosulfan, Gills, Lindane Hepatocytes, Labeo Rohita, Hepatocytes, Kidney.

I. INTRODUCTION

In order to achieve the ultimate goal of food security for increasing population, post-independent India saw a new upsurge in the form of famous ‘Green Revolution’. In this process of development, chemicals like pesticides/insecticides have become an important tool to protect food grains and other agricultural products against pest. Their indiscriminate use not only affect the target organisms but also extend their toxic effects to the non-target species (Pimentel, 1971; Eicher et al., 1978). The consumption of pesticides increased several hundred folds (from 154 ton in 1953-54 to 80,000 ton in 1994-95). But (in 2003-2004) its consumption showed a decline primarily due to the restrictions imposed on the use of certain pesticides (Samanta, 2007). Insecticides constitute about 75% of the total pesticides used.

Among the various insecticides groups, organochlorines are widely used in agriculture and insect control / malarial control programmes. These chemicals are not preferred because of their persistence in the environment and are also called as ‘hard pesticides’. These organochlorine insecticides can also be classed as ‘neuropoisons’ because they target the nerve membrane and in addition to this they disrupt the ionic balance of non-neuronal cells (Davis and Wedemeyer, 1971; Ghose, 2005). Not only parent pesticides but their metabolites also exert more deleterious effects e.g. the metabolized products of Endosulfan (endosulfan sulfate, endosulfan idol, endosulfan ether, endosulfan hydroxyether and endosulfan lactone) (Khan et al., 1979; Bend and James, 1979; Dreher and Podratzki, 1988). The ultimate destination of all these pesticides and their residues from agricultural fields and industries are aquatic ecosystems (ponds, lakes and rivers). The present study has been conducted with an aim to investigate the effect of organochlorine pesticides i.e. Endosulfan and Lindane on histology of liver, gills and kidney tissues.

II. MATERIAL AND METHODOLOGY

During present studies, fish *Labeo rohita* (Hamilton) varying in length from 19.9 cm – 21.2 cm and weight from 70.8 g to 90.45 g were brought from fish farm to the laboratory and acclimatized in plastic tubs containing dechlorinated tap water for 15 days. During acclimatization, fishes were fed *ad libitum* and water of tubs was renewed daily.

Experimental setup for determination of LC50 value.

The static test technique (Doudroff et al., 1951) was employed for 96 hour bioassay. On the basis of preliminary tests, the LC_{50}/96h was found to be 0.12 ppm for Endosulfan and 2.6 ppm for Lindane.

Sublethal treatment protocol

The LC_{50}/96h values of both pesticides (Endosulfan and Lindane) were kept at standard and various dilutions (i.e. 10%, 20% and 30%) were made from the standard solutions. The fish were divided into following groups-

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control ‘C’ (without pesticide)
EI (10% of Endosulfan)
EII (20% of Endosulfan)
EIII (30% of LC₅₀/96h for Endosulfan)
LI (10% of LC₅₀/96h for Lindane)
LII (20% of LC₅₀/96h for Lindane)
LIII (30% of LC₅₀/96h f o r Lindane)

The total experimental period was of 45 days and run in duplicate.

Histological studies
The gills, liver and kidneys tissues collected were fixed in Bouin’s fixative. After post fixation treatment following routine dehydration and clearing, tissues were embedded in paraffin wax (congealing point between 58°C – 60°C). The 5-8µm thick sections of the tissues were cut on a rotary microtome. The same were stained with haematoxylin–eosin stain and mounted in DPX.

Physico-chemical characteristics of water
Water from each tub was analysed for different physico-chemical parameters following the methods as shown against each.

i. Water Temperature
   - Mercury bulb thermometer

ii. pH
   - Hanna pH meter

iii. DO
   - APHA (1985)

iv. Electrical conductivity
   - Conductivity meter

v. FCO₂
   - APHA (1985)

III. RESULTS

LIVER
The toxic effects of Endosulfan in the liver of all exposed groups (EI, EII, EIII) revealed a dose-dependent histopathological changes.

On 30th day, diffuse congestion and haemorrhages of blood vessels, dilated and engorged sinusoids, micro to macrovacular degeneration of hepatocytes and bile duct hyperplasia (Plate 1, Figs. b, c, d; Fig. c) were seen.

On 45th day, all the Endosulfan treated groups recorded multifocal necrosis, mononuclear cell infiltration, cytolytic changes of haemopoietic tissues and bile ductular hyperplasia with periportal fibrosis. Coagulative necrosis of hepatocytes were also observed (Fig. d)

The toxic effects of Lindane in low concentration group i.e. LI revealed diffuse dilation and engorgement of blood vessels, microvacuolar degeneration with eccentric nuclei of hepatocytes (Plate 3, Fig. a) on 30th and 45th day of sacrifice. The mid concentration group i.e. LII showed congestion, haemorrhages, micro to macrovacular degeneration of hepatocytes (Fig. e) and mild mono nuclear cell infiltration in the periportal area.

On the contrary, the high concentration exposed fishes i.e. LIII revealed macrovacuolar degeneration, multifocal necrosis (Plate 3, Fig. b), periportal inflammation with mononuclear cell infiltration, bile duct hyperplasia (Plate 3, Fig. c) and periductal fibrosis (Plate 2, Fig. d) on 45th day of sacrifice.

GILLS
On 30th day, the gills of Labeo rohita exposed to low (EI) and mid concentration of Endosulfan (EII) recorded a severe congestion and hemorrhages of capillaries, ruptured blood vessels and clumped red blood corpuscles with thrombus formation (hematocele). On 45th day, in addition to above, degeneration and necrosis of epithelial cells and fusion & atrophy in secondary lamellae were seen (Plate 2, Fig. f; Plate 4, Fig. f)

The higher concentration group (EIII) of Labeo rohita revealed loss of cellular elements and leaf like primary lamellae with central cartilage body (Plate 4, Fig. a). The distal stem portion of primary lamellae revealed diffuse necrosis with infiltration of eosinophilic granular cells.

On 30th day, the lindane exposed groups i.e. LI and LII recorded diffuse congestion and hemorrhages of blood capillaries and microeosinophilic bodies with clumping of erythrocytes (Plate 3, Figs. b & c).

On 45th day, the secondary lamellae demonstrated severe hemorrhages with mononuclear cell infiltration and with multifocal necrosis of lamellae (Plate 3, Fig. d).

In addition to congestion and hemorrhages, the higher concentration group (LIII) fishes exhibited a severe degeneration and necrosis of primary and secondary gill lamellae. In some instances the secondary lamellae were severely fused which may be due to necrosis of epithelial cells. There was also severe loss and necrosis of pillar cells (Plate 3, Figs. e & f).

KIDNEYS
The low (EI) and mid concentration (EII) groups revealed severe swelling and degeneration of proximal and distal convoluted tubules on 30th day. On 45th day, in addition to above changes there were narrowing of renal tubules and sometimes loss of haemopoietic tissue (Plate 4, Fig. c). The glomeruli also showed severe enlargement with hypercellularity in them.

On 30th day, the high concentration group (EIII) revealed severe congestion and hemorrhages of blood vessels, perivascular fibrosis, micronuclear cells infiltration, severe degeneration and necrosis of renal tubules and atrophy of glomeruli (Plate 4, Fig. d). On 45th day, the severity of lesions were highly pronounced with perivascular fibrosis, degeneration and necrosis of collecting tubules (Plate 4, Figs. e & f). The dead and desquamated
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epithelial cells were seen in the collecting tubules (Plate 5, Figs. a & b) at higher concentration of Endosulfan.
The low and mid concentration groups of Lindane exposed fishes (LI and LII) demonstrated a mild congestion and haemorrhages of blood vessels after 30th day of exposure. On 45th day of sacrifice, these groups showed a mild tubular degeneration but the fishes treated with higher concentration of Lindane (LIII) showed diffuse swelling and degeneration of proximal and distal convoluted tubules and there was also mild to moderate cytolytic necrosis of haemopoietic tissue (Plate 5, Figs. a & b).

PHYSICO-CHEMICAL CHARACTERISTICS.
During the present study, the parameters analysed were-
temperature (26.6°C-28.8°C)
dissolved oxygen (5.6-6.6 mg/l)
PH (7.06-8.03)
electrical conductivity (291.3-418.0μs/cm) and
free carbon dioxide (3.0-4.2mg/l).

IV. DISCUSSION
Liver due to its position, function and blood supply, is one of the organs most affected by contamination in water (Camargo and Martinez, 2007). Impaired blood supply reported to be a causative factor of necrosis (Mohamed, 2009). Vacuolation in hepatocytes of freshwater fish after pesticides poisoning was a result of depleted glycogen and lipid (Agarwal and Srivastava, 1980). The hepatic vasodilation probably acting as a device for greater transport of pesticides and suggests that liver helps in detoxification. Moderate to marked cellular infiltrations which comprised mostly of mononuclear cells in the liver of exposed Labeo rohita explained it as a defence mechanism in the fish to counter the toxic metabolites (Das and Mukherjee, 2000). Severe damage especially in the form of duct hyperplasia and periductular fibrosis suggests pesticides are excreted in the bile after intoxication. Stasis of blood may be responsible for the cellular degeneration and necrosis in liver of fish.
The histomorphological alterations recorded in our present findings reduced the lamellar area which decreased the capacity of gas exchange across gills. The lamellar fusion may be
considered as reaction to the pesticidal intake or an adaptive response to prevent the entry of xenobiotics/pesticides/pollutants through the gill surface. It also resulted in the increase of the distance between the external environment and blood and thus serves as a barrier to the entrance of pollutants (Poleksic and Mitrovic-Tutundzic, 1994; Fernandes and Mazon, 2003). The observed cellular damage in terms of necrosis can adversely affect the gas exchange and ionic regulation (Dutta et al., 1993).

The deformities observed in present findings such as necrosis of renal tubules, dead and desquamated epithelial cells, atrophy of glomeruli and hemorrhages suggested that pesticides enter the kidney and disrupt their normal functioning by histopathological alterations of kidney tissue. The necrosis of renal tubules affects the metabolic activities and promote metabolic abnormalities in fish (Yokote, 1982).

CONCLUSION

The histopathological changes recorded in the liver, gills and kidneys of Labeo rohita very clearly indicated that these pesticides strongly affects the health of food fish. It is thus suggested that care must be taken not to allow the entry of such chemicals/pesticides into the habitat of the fishes.

BIBLIOGRAPHY


