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IMPACT OF SUBLETHAL CONCENTRATION OF ENDOSULFAN ON BIOCHEMICALS AND HISTOLOGY OF ORGAN TISSUES OF FRESHWATER FISH, *LABEO ROHITA* (HAMILTON, 1822)

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ABSTRACT
Exposure of the freshwater fish, *Labeo rohita* to sublethal concentration (0.05 ppm) of endosulfan over a period of 96 (hrs.) has revealed that the organochlorine pesticide had a profound effect on the total content of carbohydrates, lipids and proteins in the muscle tissue (mg%) and proteins in the liver tissues. Histological observations of the experimental liver, kidney and gills have shown pathological lesions leading to necrosis of hepatocytes, glomorulus of kidney and primary and secondary gill filaments of gills, which was on par with the duration of exposure of fish to endosulfan and thereby affect homeostasis of fish, *L. rohita*.

INTRODUCTION

Cultural evolution has lead to the use of pesticides for the control of pests and in turn brought the pollution of aquatic systems. One among such pollutants is the endosulfan which is a persistant and hazardus agent (Keith and Telliard, 1979; Holden, 1992). Endosulfan has equal toxicity to insects, birds, mammals, fish and aquatic life (Ware, 1975; Das, 1998; Das and Mukherjee, 2000). *L. rohita* is one of the Indian major carps that dominate freshwater aquaculture in India and hence described India as 'carp country'. As fish is a staple food for human and prey for carnivores, it is a component of food chain (Deichmann *et al*., 1975.). The bioaccumulation of endosufan on these animals would affect the homeostasis and result in the biomagnification. This could affect the economic growth of our country, as well as scarcity of food resources and outbreak of different type of ailments in human population and animals (Thomas and Indra, 2008). Hence the present work is undertaken to find out the impact of sublethal concentration of endosulfan on the biochemicals parameters viz., carbohydrates, lipids and proteins and on the histology of organ tissues like muscle, gills and liver of the freshwater fish, *L. rohita*.

MATERIALS AND METHODS

The fresh water fish, *Labeo rohita* were obtained from the fish farm at Thirumanur, Thanjavur. They were transported in aerated polythene bags and acclimatized to laboratory condition for a period of ten days. Fishes measuring 9.6 $\pm$ 0.158 cm in length and weighing 25 $\pm$ 1 g were used for the present work.

Exposure of endosulfan: Fishes were grouped into control and experimental which were exposed to sub lethal concentration of endosulfan (0.05 ppm), over a period of 96 h.

Biochemical estimations: Total content of carbohydrates was estimated by anthrone method (Roe, 1955), lipids by Folch *et al.*, (1957), and proteins using Biuret method (Gornall *et al.*, 1949).

Histology: Tissue samples were fixed in Bouins fixative and processed for microtechnique. The gills were treated in 5% nitric acid in 70% alcohol for 24 h in order to soften the skeleton. 5 μm sections were stained in Ehrlich's (H&E) stain and observed under Carl Zeiss microscope for histopathology and photographed.

RESULTS

Biochemical analysis of muscle tissues of the endosulfan exposed fish showed a decrease in the total content of carbohydrates and lipids by 44.3 and 37.3 (%), respectively, when compared with the control. Where as that of protein in the muscle as well as liver tissues showed an increase of 45.93

and 91.76 (%), respectively (Table 1).

Histological observations of liver tissue from control fish showed normal homogeneous mass of hepatocytes with centrally located nuclei and granular cytoplasm. Bile canaliculi opens into the hepatic ducts and hepatocytes are supported with a fine network of connective tissue (Fig.1a1).

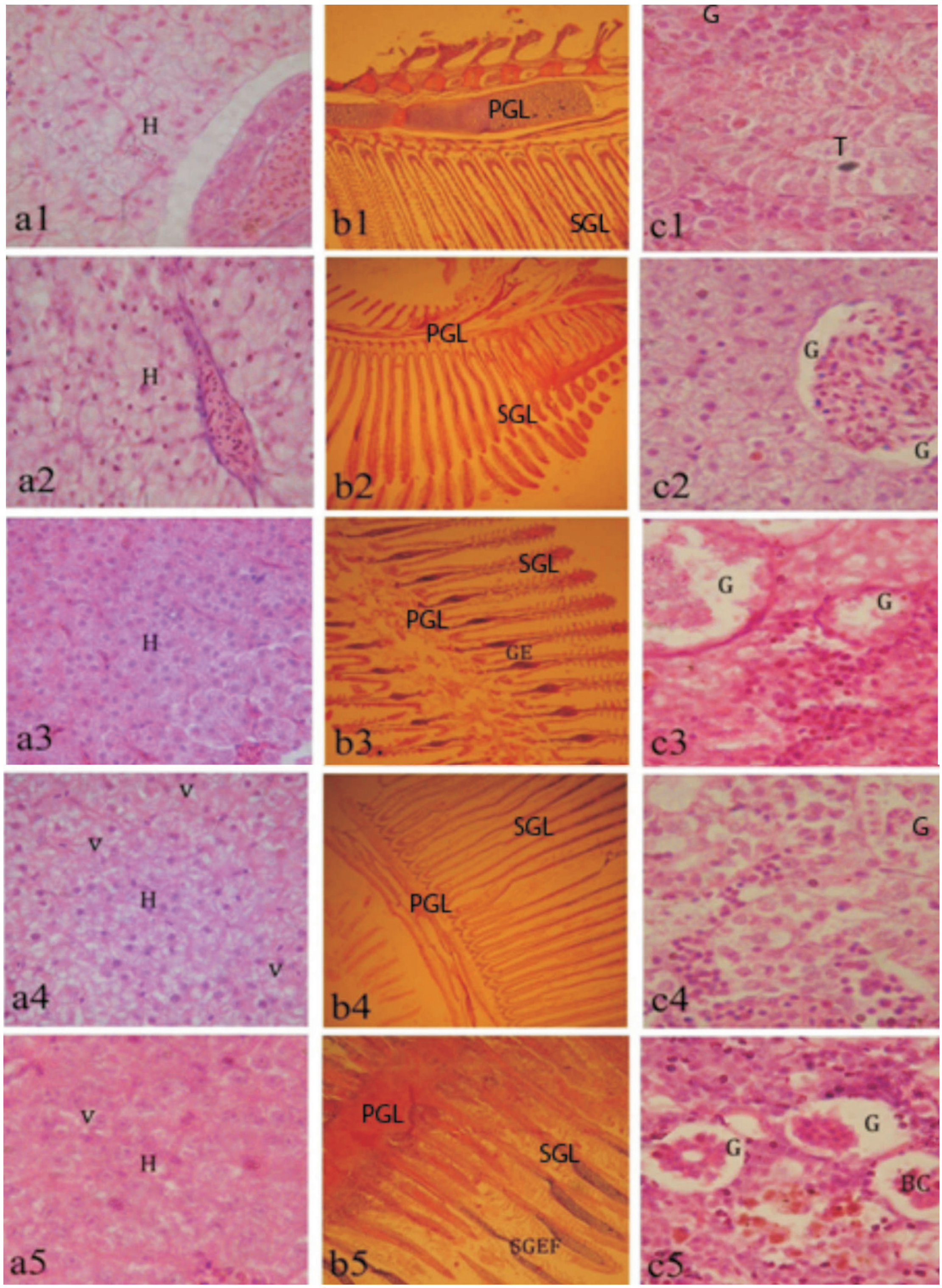

Figure 1: Histological sections of liver, gills and kidney of of L. *rohita*

a- Liver sections (H and E stained, X100), **1**-control, **2**(X50), **3, 4** (X50) and **5** are endosulfan exposed for 24, 48, 72 and 96 hr, respectively.; **b**- gills sections (X1000); **c**- kidney sections (X1000). H- Hepatocytes, T- Tubules, V- Vacuoles, GE-Swelling of gill epithelium, PGL-Primary gill lamellae, SGL- Secondary gill lamellae, SGE F-Fusion of secondary gill lamellae, G- Shrinkage and degeneration of glomeruli, BC- Space in Bowman's capsule.

Table 1: Total content of biochemicals (mg%) in the muscle and liver tissues of *L. rohita*

Samples	Muscle	Liver		
	Carbohydrate	Lipid	Protein	Protein
Control	0.413 ± 0.05	2.60 ± 0.06	2.09 ± 0.07	1.82 ± 0.02
Treated	0.230 ± 0.11	1.63 ± 0.08	3.05 ± 0.06	3.49 ± 0.04
Decrease or Increase(%)	44.30↓	37.30↓	45.93↑	91.76↑

Parenchymatous distribution of the hepatocytes in cords around the sinusoids was also seen. However, endosulfan treated liver tissues revealed compactly arranged hepatocytes but with a strong cytoplasmic vacuolization with increased basophilia suggestive of chronic toxicity and with eccentric or pyknotic nuclei. Hepatocellular necrosis with parenchymal vacuolization, breakdown of cell boundaries, swelling and degeneration of the endothelial lining cells leading to the damage of central veins were also observed with increase in the duration of exposure to endosulfan (Fig.1a2 to 1a5).

Histological observation of gills showed degenerative changes when compared with the control tissue (Fig. 1b1). Primary gill lamellae had mild congestion of blood vessels, fusion of lamellae and marked hyperplasia of the branchial arches. Dilatation of blood capillaries, hyperplasia of the epithelial lining, abnormal raising or swelling of epithelium, necrosis and shortening as well as fusion of the secondary lamellae were also observed. The primary gill lamella is lined by a thick stratified epithelium that contains numerous mucous and chloride cells responsible for excessive mucus secretion (Fig. 1b2-b5).

Control kidney sections had normal characteristics (Fig. 1c1), whereas endosufan exposed kidney sections showed disintegration of convoluted tubules with large intra-cytoplasmic vacuoles in the epithelial cells and lumen with invariably in-filtered mononuclear cells. Shrinkage and degeneration of the glomeruli, increase of space within the Bowman's capsule were also seen (Fig. 1c2-c5).

DISCUSSION

A significant decrease in carbohydrate content of the muscle tissue in the present study is similar to the earlier observations in the tissues of muscle, liver and gills in *L. rohita* treated with monovalent mercury (Rajasubramaniam *et al*., 2006), *Oreochromis mossambica*, treated with methyl parathion (Sivaprasada Rao and Ramana Rao, 1979), *Anabas candeng* treated with zinc (Natarajan, 1982, 1983). The energy yielding carbohydrate metabolism is disturbed when the living organisms are subjected to pollutants (Grant and Schoettger, 1972; Mcleay and Brown, 1974; Srivatsava *et al*., 1977; Sastry and Sidiqqui, 1982; Simon *et al*., 1983; Metelev *et al*., 1983). Insecticides were known to induce hyperglycemia of blood in *Clarias batrachus* (Mukhopadhya and Dehadrai, 1980).

Quantitative increase of protein content in the hepatic tissues has been reported in the fish *Channa punctatus*, when exposed to organophosphorous pesticides (Anees, 1974, 1978) and dieldrin induced rat liver (Bhatia *et al.,* 1973). Tripathi and Verma (2004) suggested endosulfan-induced impairment of metabolism in fish, *Clarias batrachus*, which appeared to be due to inhibition of transcription at some unknown points and 40% reduction in activities of CS and G6-PDH in liver tissues.

Histological studies on the effect of endosulfan on liver tissue confirm similar toxicity to that of lindane in *Cyprinus carpio* (Mc Donald, 1983). Appearance of many small vacuoles in the hepatic cells was reported in brown trout fry and adult guppies when exposed to DDT and this they relate to resorption of fatty yolk (King, 1962) and the degree of structural heterogeneity is enhanced with increasing concentration of toxicants (Hawkes, 1980). Wide variety of insecticides and other toxic by-products tend to accumulate in high concentration within the liver (Hinsen *et al*., 1971; Metelev *et al.,* 1983) as liver is the major organ for detoxification (Dutta *et al*., 1993). Irregular nuclear profiles with scalloped edges, necrotic changes in nuclei, acute toxicity and cell death have been reported in fish when exposed to pesticides. Glover *et al.,* (2007) have reported a significant activity of liver 7-ethoxyresorufin-*O*-deacetylase upon exposure of endosulfan (710 μg kg^{-1}) by day 35 whereas other hepatic indicators of stress impacts and responses viz., glutathione-*S*-transferase and glutathione peroxidase activities and hepatic á-tocopherol content remained unchanged. In the liver the primary effects were glycogen depletion and lipidosis and these changes were typical of a generalised stress response. They also suggest that histology endpoints may prove to be the most sensitive indicators of dietary endosulfan exposure, the organismal relevance of these structural changes must be considered in the absence of effects in other biomarkers at dietary levels less than 710 μg kg^{-1}.

Gills are the primary route for the entry of pesticide (Mallatt, 1985; Richmonds and Dutta, 1989). Peal dace and fathead minnows when exposed to polluted environment have exhibited necrosis and rupture of gill epithelium leading to hypoxia and respiratory failure (Leino *et al.,* 1987). Excessive secretion of mucus, lifting of the gill epithelium and the fusion of gill lamellae could be protective mechanism so as to reduce the vulnerability caused by the pesticide (Mallatt, 1985). Riji John and Jayabalan (2007) have observed hyperplasia, lamellar fusion, curling and bulging of tips of primary gill lamellae, exudation of erythrocytes, when the fish *Cyprinus carpio* was exposed to sublethal concentration of endosulfan.

The kidney of fish receives much of the largest portion of postbranchial blood and therefore renal lesions might be expected to be a good indicator of environmental pollution. Tubular degeneration, intertubular and intratubular deposits in catfish, *Ictalurus punctatus* upon exposure to methyl mercury (Kendall, 1975). *Notopterus notopterus* upon exposure to sublethal concentration of phenolic compounds exhibited degeneration and dissolution of epithelial cells of renal tubules, hypertrophy and necrosis (Gupta and Dalela, 1987). Similar observations were reported in *Cyprinus carpio* when exposed to Anthio 40 EC, Stox and Basuden 10G, organochlorine and organophosphate compounds (Csepai, 1978). However our observations have revealed a time dependent shrinking and degeneration of the glomerulus, and vacuolization of epithelial cells of uriniferous tubules with sublethal dose of endoulfan. Hence it is evident that endosufan had a profound effect on the biochemicals as well as on the histology of tissues under investigations that brought imbalance in homeostasis of *L. rohita*.

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