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Impact of exposure to sub lethal concentration of Monocrotophos on blood parameters, biochemical constituents and histology of major carp, *Catla catla* (Hamilton)

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ABSTRACT The major carp, *Catla catla* on sub lethal exposure to monocrotophos for 30 days revealed significant decrease in blood parameters like RBC, WBC & Hb. As a result of chronic exposure the organic constituents like total free sugars, cholesterol and protein of liver and muscle also decreased significantly. Remarkable histological changes have also been observed in liver and gill of experimental groups. The reason for the significant reduction in blood parameters, tissue organic constituents and the histopathological changes is discussed.

(**Keywords**: *Catla catla,* monocrotophos, blood parameters, tissue organic constituents, histopathology)

INTRODUCTION

In modern agricultural practices organophosphorous compounds are widely used as powerful insecticides. Pesticides become readily available in the food chain and subsequently bioaccumulate in both aquatic and terrestrial flora and fauna. Organophosphate such as monocrotophos is highly persistent, bioconcentrate in food chains and can severely affect whole populations or species of wildlife, has led to ban and restriction in use [1].

The effects of pollutants on a population can be better understood and predicted by studying the sub-lethal effects on the individual or by focusing on processes at lower level of biological organization. The residual effects of pesticides can be studied in important organs like liver and gills damaged in fishes exposed to pesticides [2]. Hematological studies can be used to provide useful information about the severity of stress response.

The fish exposed to an organophosphorous insecticide showed elevated levels of cholesterol, total lipids and total protein in liver and muscle [3]. Behavioral changes which reflect the physiological status and condition in fishes have been recorded when the fishes are exposed to pesticide stress.

A careful perusal of the literature reveals not much of work has been done on monocrotophos, an organophosphorous widely used in the fields of Tiruchirappalli district; hence an attempt has been to study the impact of sub lethal concentration of monocrotophos on blood parameters, tissue organic constituents and histopathology of liver and gill of the major carp, *Catla catla* (Hamilton).

MATERIALS AND METHOD

The major carp, *Catla catla* was collected from the fish farm at Poyakudi of Tiruchirappalli district. The length and weight of the fish ranged from 15.0 to 19.0 cm and 45.00 to 90.00 g, respectively. In the laboratory, the fish were maintained in a large circular tank for two weeks. During this period they were fed with pelleted (rice bran & oilcake) feed on alternate days and the water was renewed daily.

The temperature, pH, salinity and dissolved oxygen of the water were maintained at $27^0C \pm 1^0C$, 7.55 ± 0.1, $0.76 \pm 0.09‰$ and 7.20 ± 0.12ml/l. After the acclimatization, fishes were divided into two groups of 5 each and transferred to two rectangular tanks filled with 40 liters of well water each. One of the tanks served as control and the other as experiment. In this study, one of the widely used organophosphate insecticides, Monocrotophos commercial grade (w/v) was used at a sub lethal concentration of 0.01ppm [4].

To obtain the sub lethal concentration of 0.01ppm of Monocrotophos, one ml of commercial preparation monocrotophos [1 mg of the active principle [(dimethyl (E)-1-methyl-2-(methylcarbamyl) vinyl phosphate)] was mixed with 40 liters of water. Then the water in the tank was mixed well, this would yield 0.01ppm of the pesticide in the medium. The

present study includes a chronic period of 30 days exposure to sub lethal concentration of monocrotophos. From the laboratory acclimatized fishes, two groups of 5 fishes each were selected in the same size range and then introduced into two tanks measuring 45 x 48 x 25 cm with 40 liters of well water.

One of these served as control and the other as experimental. To the experimental tanks was added 1 ml of monocrotophos solution, and mixed well. During the chronic study, the fishes were fed with pellets on alternate days and the water was also renewed on alternate days an hour after providing the food. At the end of 30^{th} day the blood and the tissues were collected individually and used for analysis. Samples of blood were collected by sievering the caudal region of each and every fish and total erythrocyte count (RBC), total leucocytes count (WBC) and the haemoglobin concentration were determined by the standard procedures [5].

The biochemical analysis of total free sugars, cholesterol and protein were determined tissues following the procedure of Roe (1955) [6], Zarrow et al (1964) [7] and Gornall *et al* (1949) [8]. The tissues proteins qualitative changes level was determined using SDS PAGE have been reported [3, 9]. The histological studies on liver and gills were done after fixing the tissues in bouins fluid by standard procedures [10]. The sections were photomicrographed using Canon T-70 camera. The statistics analyses of the data were done as per the procedures [11].

RESULTS

No fish deaths occurred in the control group or fish exposed to sub lethal concentration of monocrotophos for 30 days. The mean level of RBC count decreased from 5.09 ± 0.25 millions/mm^3 to 3.02 ± 0.23 millions/mm^3. The mean level of haemoglobin decreased from 6.2 ± 0.4 g% to 4.86 ± 0.36 g%. The mean level of WBC count decreased from 6.22 ± 0.36 to 5.17 ± 0.57 thousands/mm^3 due to the insecticidal stress (Table 1).

The mean liver total free sugar level in control group was 135.81 ± 11.65 mg/g. wet wt. Where as in experimental group the mean level decreased to 94.22 ± 7.84 mg/g wet wt.

The decreased liver cholesterol level in that mean from 80.33 ± 9.31 mg/g wet wt to 69.67 ± 6.56mg/g wet wt. The mean liver protein also decreased from 26.03 ± 2.36 to 21.41 ± 1.95 mg/g wet wt mg/g wet wt (Table 1).The mean muscle total free sugar level in control group was 67.95 ± 5.54 mg/g. wet wt. Where as in experimental group the mean level decreased to 47.25 ± 4.48 mg/g wet wt. The decreased muscle cholesterol level in that mean from 30.32 ± 4.39 mg/g wet wt to 20.74 ± 1.98 mg/g wet wt. The mean muscle protein also decreased from 71.63 ± 7.91 to 48.99 ± 3.94 mg/g wet wt mg/g wet wt (Table 1, Fig 1 and Fig 2). The SDS PAGE analysis of *liver* and muscle proteins *Catla catla* revealed the presence of highly expressed subunits of approximately 17.5 KDa and 18 KDa.

The liver of control fish appear to be normal (Figure 3). The T.S. of the liver showed a continuous mass of hepatic cells which are large, hexagonal in shape with more or less centrally placed nucleus. But in exposed fish, the liver cells are enlarge with vacuolation and revealed damaged islets of Langerhans with cloudy swelling of cells. Biliary hyperplasia was observed at certain regions of the hepatic tissue. This might be indicating the regenerating hepatic cells withstand the toxic stress conditions.

Thirty days exposure to sub lethal concentration of monocrotophos produced considerable histopathological changes in the gills of *Catla catla* exposed group when compared to control group as shown in (Figure 4). The present study also revealed higher production of mucus. The tip of the primary lamellae was shapeless and seemed to be encoded. The secondary lamellae varied in shape widely. Debris of interlamellae damaged secondary lamellae and red blood cells were often noticed between secondary and primary lamellae. Degeneration of cartilaginous cells was also observed in treated gills.

DISCUSSION

In the present fish under chronic exposure to monocrotophos showed a significant fall in RBC and WBC count and haemoglobin content. A reduction in haematological values, indicated anemia in the pesticide exposed fish may be due to erythropoiesis, haemosynthesis and osmoregulatory dysfunction or due to an increase in the rate of erythrocyte destruction in haematopoietic organs [12, 13].

Table 1: Showing alterations in blood parameters and organic constituents of tissues in control(**C**) and experimental (**E**) fishes of the major carp, ***Catla catla*** (Hamilton) on exposure to Monocrotophos for 30 days.

Nature of tissue	**Hematological parameters**					
	RBC X 10^5/mm^3		WBC X10^4 /mm^3		Haemoglobin (g %)	
	C	**E**	**C**	**E**	**C**	**E**
BLOOD	5.09±0.25	3.02±0.23	6.22±0.36	5.17±0.57	6.2±0.4	4.86±0.36
Nature of tissue	**Biochemical parameters**					
	Total Free Sugars (mg/g wet wt)		Cholesterol		Protein	
	C	**E**	**C**	**E**	**C**	**E**
LIVER	135.81±11.65	94.22±7.84	80.33±9.31	69.67±6.56	26.03±2.36	21.41±1.95
MUSCLE	67.95±5.54	47.25±4.48	30.32±4.39	20.74±1.98	71.63±7.91	48.99±3.94

The present study also revealed a significant reduction in the level of total free sugars of liver and muscle. This is in agreement with earlier reports [14, 15]. The reason for the significance decreases in tissue sugar may be due to its utilization to meet the insecticidal stress. The protein content of liver and muscle also decreased significantly due to the insecticidal stress. When exposed to sub lethal of fenvalerute technical grade and 20% EC, the total protein content was found to decrease [16, 17]. The decreasing trend in tissue cholesterol level has been observed in the present studies. In the normal animal cholesterol synthesis in liver is found to operate at a level below maximum due to continuous absorption of cholesterol from the digestive tract and to negative feedback effect. The decrease in cholesterol of liver and muscle such as its supplementation in energy yielding process of the fishes under the stress.

In the present study hypocholestremia was noted in the muscle and liver of fish *Catla catla* under monocrotophos, exposure as reported [18, 19, and 20]. The reduced cholesterol level may be due to the inhibitions of cholesterol biosynthesis in the liver or due to reduced absorption of dietary cholesterol as reported [21,22] or an increased breakdown of cholesterol levels to free fatty acids to be used in carticosteridogenesis or decreased de novo synthesis as pointed out [23]. The pesticide induced protein decrease in the tissue has been reported by earlier authors [24, 25, and 26]. The authors have suggested that the decrease in protein content of tissue may be due to its degradation and the product. The present study reveals the quantitative and qualitative change in tissue protein (Figure 1 & 2).

These reactions may be fed into TCA cycle through amino transferees to cope up with the high energy demand posed by the insecticide stress. Further, decreased protein level may be attributed to stress mediated immobilization of these compounds to fulfill an increased element for energy by the fish to cope with environmental condition exposed by the toxicants [12]. The depletion in total protein content may be due to augmented proteolysis and possible utilization of their product for metabolic purposes. On the other hand Neff [27] has opined that decline in protein content may be related to impaired food intake, increased energy cost of homeostasis, tissue repair and detoxification mechanism during stress.

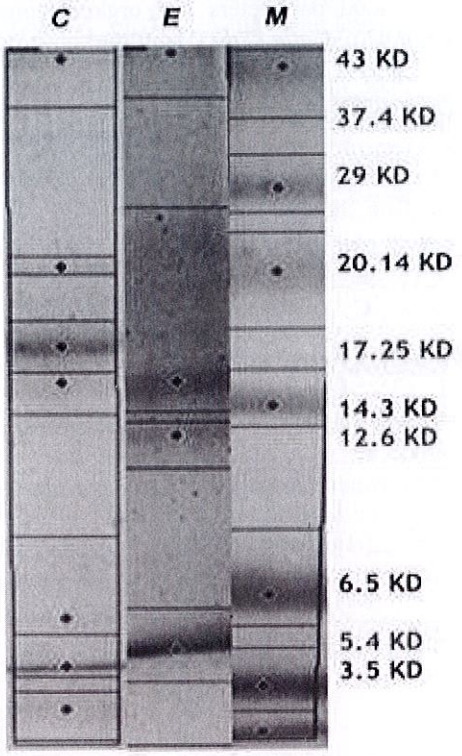

Figure 1: Protein profile of monocrotophos affected *catla catla* muscle tissue. C-Control, E-Experiment, M-Marker

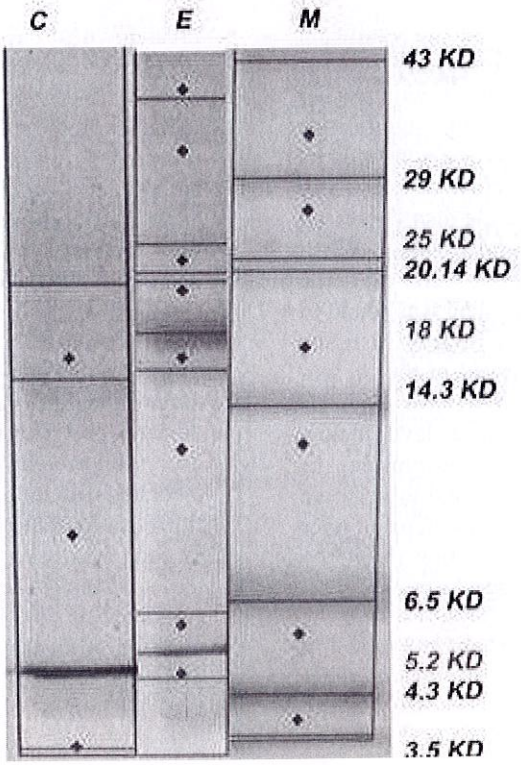

Figure 2: Protein profile of monocrotophos affected *catla catla* liver tissue. C-Control, E-Experiment, M-Marker.

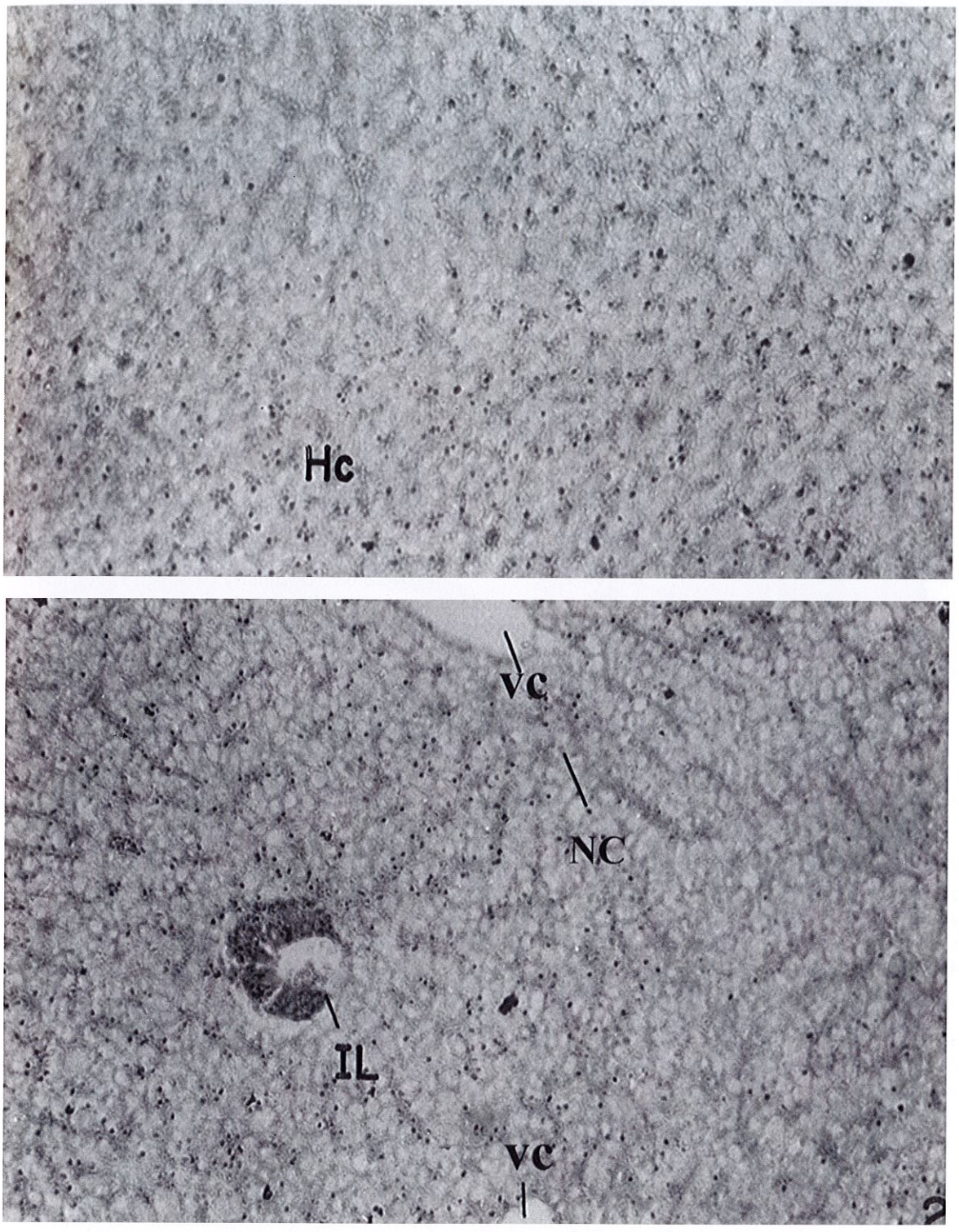

Figure 3: T.S of treated liver of catla catla showing compact hexagonal cells (HC) X 100 T.S of treated liver of catla catla showing cloudy swelling, vaculation of hepatic cells, destruction and separation of islets of langerhans, X 100 (HC =Hepatic cells, NC= Necrosis, VC= Vacuaation, IL, Islets of Langerhans.

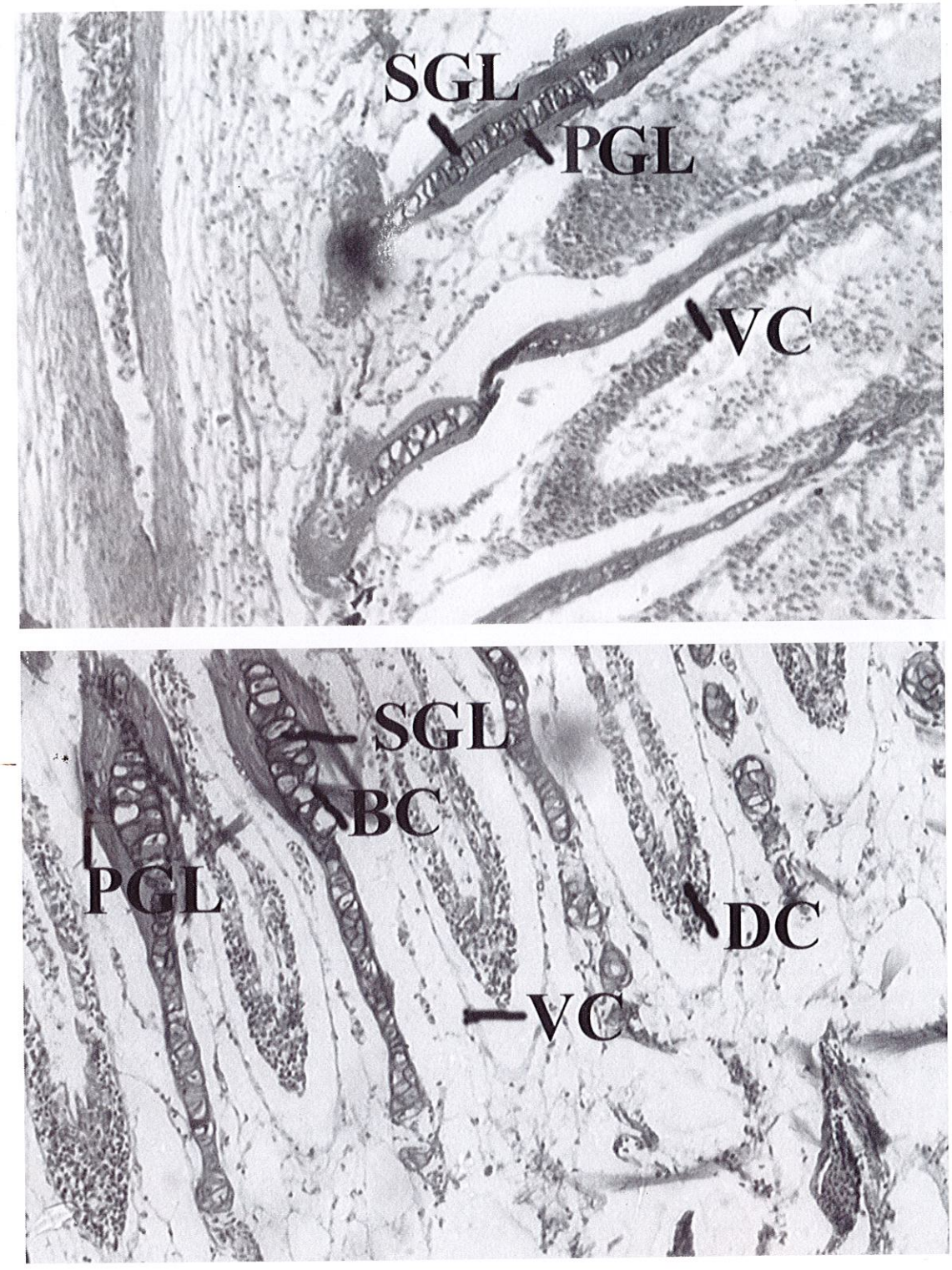

Figure 4: T.S of control gill of catla catla, showing normal vacuaation with primary gill lameliae and second gill lamellae. X 100. T.S of experiment gill of catla catla showing disintegrated cell.

After 30 days of long term exposure to monocrotophos, resulted in necrosis of hepatic tissue. Completely vacuolated areas were observed with fat deposition. Biliary hyperplasia was observed at certain regions of hepatic tissue. This might be indicating the regenerating hepatic cells to

withstand the toxic stress condition. These observations have been reported [25, 28]. Gills become edematous with prominent clubbing. Separation of primary gill lamellae and hemorrhage in the blood vessels outside the secondary gill lamellae were observed. Hyperemia of the gill filaments that engorged with blood vessels appeared.

Hyperplasia was observed in secondary gill lamellae, which led to fusion of adjacent primary and secondary gill filaments. This histopathological change observed in the gills *Catla catla* in the present study is in good agreement with the reports of [29].

CONCLUSION

As a result of monocrotophos exposure the fish showed, degradation of metabolic changes resulting in histopathological alteration in gill, liver and testis which will ultimately affect in nutritive value and reproductive capacity of the commercial important fish, *Catla catla* (Hamilton). So care has to be taken to prevent the contamination of aquatic bodies even with low levels of the insecticide monocrotophos.

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