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Hematological Responses of the Common Carp, *Cyprinus carpio* L. Exposed to the Pesticide Endosulfan

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Abstract

Common carp, *Cyprinus carpio* L., were exposed to 5%, 10% and 15% concentrations of the 96-hour LC_{50} value of endosulfan for 7, 14 and 21 days. The fish responded with significant decrease in the levels of hemoglobin and hematocrit. While little variation in the serum protein was noticed, a significant elevation of blood glu-cose level indicated a hyperglycemic condition in the treated fish. The levels of sodium and potassium of fish blood were lower than those in the controls. However, only the decrease of potassium in relation to exposure periods (7 and 21 days) was significant.

Introduction

Toxicologists are searching for sensitive indicators of the effects on fish of aquatic pollutants in order to understand their mode of action and sensitivity and also to develop a basis for corrective action in cleaning up water bodies before the health of aquatic populations is seriously threatened. Since blood forms a unique compartment between the external and internal environments, agents including pesticides that cause stress in fish can alter the composition of the blood. Hence, the study of the hematological parameters of the fish responsive to pesticide stress is gaining recognition as we search for useful tools for monitoring the level of pollutants in the environment.

Organochlorine pesticides are widely used in Southeast Asia in rice farming (Perschbacher and Sarkar 1989). They are reported to be toxic to several nontarget organisms including fish (Holden 1973)

and also encourage epizootics of ulcerative fish diseases (Tonguthai 1985). The use of endosulfan, an organochlorine insecticide, is increasing in India due to the ban on endrin and to its comparatively lower toxicity to mammals. Further, as fish are highly susceptible to endosulfan, it is used for removal of weed fishes from ponds and small lakes before their restocking with the desired species (Gill et al. 1991). In fishes, at sublethal concentrations, endosulfan affects the reproductive physiology (Basak and Konar 1976), reduces the respiratory rate (Manoharan and Subbiah 1982), causes deformity in developing embryos (Kulshrestha and Arora 1984) and alters tissue proteins (John and Jayabalan 1993).

The present study investigates the hematological responses of the common carp *Cyprinus carpio* exposed to sublethal concentrations of endosulfan. We measured hemoglobin and hematocrit values, blood glucose and serum protein, potassium and sodium levels.

Materials and Methods

Fingerlings of *C. carpio* var. *communis* aged 90 days, measuring 12-14 cm in total length (TL) and weighing 8-9 g were obtained from the State Fisheries Farm, Badra River Project, Karnataka State, India. All the fingerlings belonged to a particular brood. They were transported in oxygenated containers with minimum stress to our College Fish Farm, then stocked in cement cisterns (5 x 5 x 1 m) fertilized with cowdung at the rate of 8,000 kg·ha⁻¹ under static water conditions for 15 days. Apart from the natural food available in the cisterns, the fingerlings were fed a mixture of rice bran and groundnut oilcake in the ratio of 1:1 by weight at the rate of 5% of body weight per day. They were then acclimated in a plastic pool of 1,500 l for a week in the laboratory. During acclimation, the fish were fed only standard pelleted feed described by Varghese et al. (1977) at the rate of 5% of body weight per day.

Fresh water drawn from a well in the college campus was used for the study. Before the start of the experiment, the water collected and stored in plastic troughs was filtered through filter paper to remove debris and then aerated. The pH of the filtered and aerated water used for the experiment ranged between 7.4 and 7.6 and the average hardness from 50 to 54 ppm CaCO₃. All the tests were performed at room temperature (26±2°C).

Endosulfan (6, 7, 8, 9, 10, 10 - hexachloro 1, 5, 5a, 6, 9, 9a - hexahydro - 6, 9-methane - 2, 4, 3 - benzo(e) - dioxathiepin - 3 - oxide) 35% EC marketed under the brand name Hexasulfan 35% EC manufactured by Bharat Pulverising Mills Pvt. Ltd., Bombay, was used.

Tests were conducted at three sublethal concentrations of endosulfan viz., 5% (0.26 ppb), 10% (0.52 ppb) and 15% (0.78 ppb) of the 96-hour LC_{50} value of 5.2 ppb (Chandrasekar 1990). For the study, 12 fish each were released into 40-l glass tanks containing 30 l of the test medium. Tests were performed in duplicate. Test media were renewed once a day and the test fish were fed daily ad libitum.

Samples of four fish each were drawn at the start of the experiment and at seven-day intervals up to the 21st day. Before being sampled for blood, the fish were anesthetized with MS-222. The blood was always drawn from the caudal vein using 1-cc disposable syringes. For the estimation of hemoglobin (Hb) content and hematocrit (Ht) values, heparinized syringes were used and blood was collected in heparinized vials. For sampling blood for glucose, serum protein and electrolytes, nonheparinized syringes were used and the blood was collected in flouride-coated bottles for blood glucose estimation, and in ordinary vials for serum protein and electrolytes (sodium and potassium). Since the blood from each fingerling was limited, blood samples obtained from several individuals were pooled and two samples were analyzed for each blood parameter in each condition.

The estimation of hemoglobin content in the blood of fish was made using the "acid hematin method" (Hesser 1960) expressed in gram per cent (g%), of volume and hematocrit values by the "micro-hematocrit method" (Hesser 1960) calculated as volume of the erythrocytes as a percentage of plasma volume (Ht%). The mean corpuscular hemoglobin concentration (MCHC) was estimated from the values of Hb content (g%) and hematocrit (Ht%) as follows:

$$\text{MCHC} = \frac{\text{Hb}}{\text{Ht}} \times 100$$

For the estimation of serum total protein, blood glucose, and serum sodium and potassium, the techniques of Varley (1988) were

followed: total protein by the Biuret method; glucose by autoanalyzer - glucose oxidase; and sodium and potassium levels by autoanalyzer - flame photometer.

Two-way analysis of variance (Snedecor and Cochran 1967) was used to test for significant differences in the values of the parameters in fish exposed to sublethal concentrations and to different exposure periods; and to distinguish which means are different from one another, Duncan's multiple range test was carried out.

Results and Discussion

In the present study, the 96-hour LC_{50} of endosulfan (Hexasulfan) 35% EC to *C. carpio* was found to be 5.2 ppb. However, Naidu et al. (1989), while using a different isomer of technical grade endosulfan (Thiodon) with 95% purity, obtained a lower 96-hour LC_{50} value of 1.93 ppb for *C. carpio*.

Hemoglobin in fish exposed to 5, 10 and 15% of the 96-hour LC_{50} for endosulfan for 0, 7, 14 and 21 days was significantly affected by both endosulfan concentration and duration of exposure (Table 1; Fig.1a). Hemoglobin decreased in proportion to concentration of the pesticide and the duration of exposure. The low levels of Hb indicated anemic conditions in fish due to stress-caused hemolysis (Panigrahi and Mishra 1978) and inhibition of aerobic glycolysis curtailing denovo synthesis of hemoglobin (Lewis 1970; Koundinya and Ramamurthi 1979; Bielinska 1987). The lower hemoglobin levels of treated fish in the present study might be due to the disruption of the iron synthesizing machinery (Beena and Viswarajan 1987).

Hematocrit values of *C. carpio* exposed to the four sublethal concentrations of endosulfan and the four exposure periods followed the same pattern as for hemoglobin content (Table 1, Fig.1b)

The Ht values decrease when a fish loses its appetite, is diseased (Blaxhall 1972) or poisoned by pesticides (Gill and Pant 1985). The reduction in the Ht values indicates that the fish suffers from anemia or hemodilution (Wedemeyer et al. 1976).

The reduction in packed cell values of *C. carpio* may also be due to increased rate of erythropoiesis as well as hemolysis as observed in the flounder *Pleuronectes flesus* when subjected to cadmium intoxication (Larsson 1975). In addition, an alteration in the

Table 1. Comparison of mean values of various blood parameters of *C. carpio* exposed to various sublethal concentrations of encosulfan for different periods.

Concentration	Hemoglobin (Hb g%)	Hematocrit (Ht %)	Protein (g:100 ml ⁻¹)	Glucose (mg 100 ml ⁻¹)	Sodium (m.eq.l ⁻¹)	Potassium (m.eq.l ⁻¹)
5% of 96-hour LC ₅₀	7.968 ^b	24.766 ^b	1.850 ^a	48.333 ^b	129.333 ^a	5.733 ^a
10% of 96-hour LC ₅₀	7.600 ^b	21.960 ^{ab}	1.933 ^a	41.666 ^a	129.666 ^a	4.933 ^a
15% of 96-hour LC ₅₀	7.133 ^a	20.660 ^a	1.800 ^a	44.666 ^{ab}	130.333 ^a	5.733 ^a
Duration						
7 days	8.300 ^c	29.246 ^c	1.766 ^a	37.333 ^a	134.000 ^a	7.300 ^b
14 days	7.733 ^b	22.163 ^b	1.850 ^a	45.000 ^b	126.666 ^a	5.500 ^{ab}
21 days	6.666 ^a	15.980 ^a	1.966 ^a	52.333 ^c	128.666 ^a	3.600 ^a

Mean values with same superscript are not significantly different (P > 0.05)

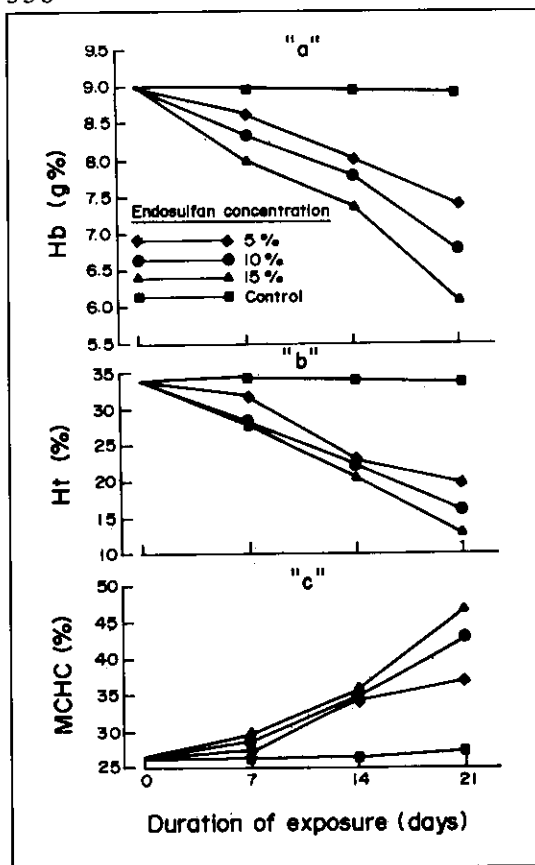


Fig.1. Hemogram of *C. carpio* exposed to four concentrations of endosulfan for four time periods. a) Hemoglobin content (Hb); b) hematocrit value (Ht); c) mean corpuscular hemoglobin concentration (MCHC).

fish metabolism would have also led to decreased values of Ht (Srivastava and Mishra 1979) in *C. carpio*. However, Naidu et al. (1989) from a 24-hour, short-term study reported an increase in the Hb and packed cell volume in *C. carpio* exposed to sublethal concentration ($1/3$ of 96-hour LC_{50} of 1.93 ppb) endosulfan (Thiodon). They stated that the fish exposed to endosulfan try to cope with the adverse condition by enhancing their respiratory capability through elevated RBC and Hb synthesis. They further inferred that, however, prolonged exposure of fish to pesticide may pose a great threat to their survival.

The MCHC value increased in treated fish compared to controls with the increase in concentration of endosulfan as well as with duration of exposure (Fig. 1c). This may be attributed to the drastic decrease in the level of Ht in treated fish caused by hemolysis.

The changes in serum protein concentration of *C. carpio* during the tests (Fig. 2a) were not statistically significant (Table 1). Changes in the fish serum proteins due to exposure to pesticides have been reported by several workers (Lone and Javaid 1976; Shakoori et al. 1976; Bano 1982).

The increases in blood glucose in fish exposed to endosulfan (Fig. 2b) were statistically significant (Table 1) for different periods

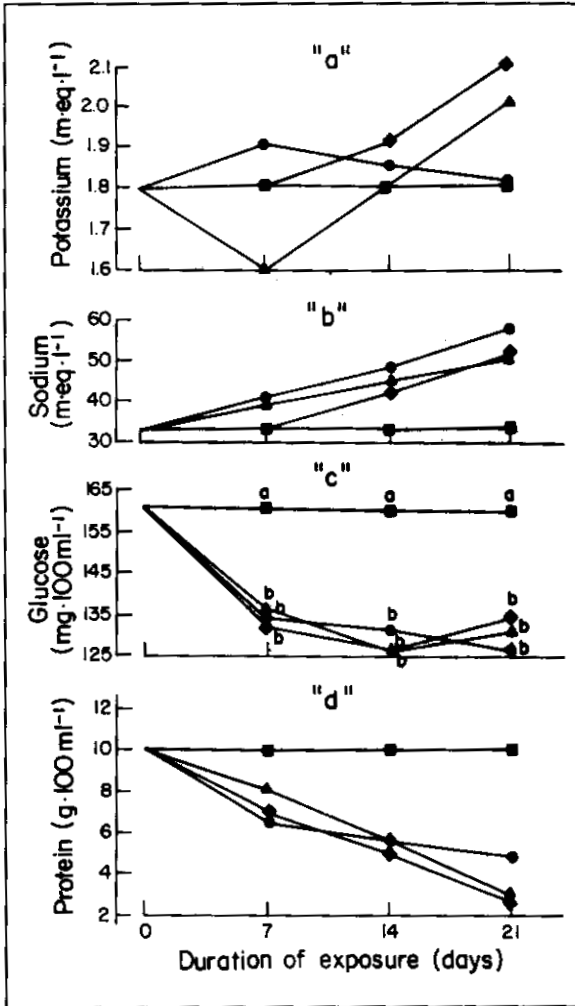


Fig.2. Alterations of blood chemical constituents of *C. carpio* exposed to four concentrations of endosulfan for four time periods. a) Serum total protein; b) blood glucose; c) serum sodium; d) serum potassium.

of exposure; however, they were significant only in 5% and 10% of 96-hour LC_{50} concentrations indicating hyperglycemic conditions. The blood glucose levels in the Indian catfish *Heteropneustes fossilis* exposed to high sublethal concentrations of endosulfan were also found to increase considerably (Srivastava and Singh 1981). The most characteristic general response to stress, from whatever source, is a pronounced rise in blood sugar level, which seem to occur whenever the physical activity exceeds what is normal for fish (Chavin and Young 1970; Wedemeyer 1972, 1973). Increase in blood

glucose level may indicate a greater energy requirement of the fish due to endosulfan stress. The increase in blood glucose level due to stress may also be viewed as a response of respiratory insufficiency (Menton 1927).

In the present investigation, the gills of *C. carpio* exposed to endosulfan were considerably damaged and this would be expected to have interfered with respiration. It is also surmised that the blood glucose rises through the mobilization of tissue glycogen particularly that of liver (Love 1970). The increase in circulating glucose levels is also related to the increase in the concentrations of circulating catecholamines or corticosteroids following stress (Fagerlund 1967; Nakano and Tomlinson 1967; Hill and Fromm 1968).

Though the concentrations of sodium (Fig. 2c) and potassium (Fig. 2d) decreased in the blood of *C. carpio* exposed to different concentrations of endosulfan for various periods, the decrease in sodium level was not significant due to concentrations and duration of exposure, and potassium level was significant for durations (7 and 21 days) of exposure only (Table 1). Eisler and Edmunds (1966) observed an increase in the concentrations of both the electrolytes in the northern puffer, *Sphaeroides maculatus* exposed to endrin. We have no explanation for the decrease in sodium and potassium levels in the blood of endosulfan-treated *C. carpio*.

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