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EFFECTS OF SUBLETHAL LEVEL OF A PESTICIDE, MONOCROTOPHOS, ON HAEMATOLOGY OF *CYPRINUS CARPIO* DURING THE EXPOSURE AND RECOVERY PERIODS

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ABSTRACT

The acute toxicity of monocrotophos on the common edible freshwater fish, *Cyprinus carpio* has been studied. The fish were procured and their sublethal, median lethal and lethal doses for 120 hours were assessed as 100 ppm, 156.92ppm and 250 ppm respectively. From the 24 hours LC_{50} and 48 hours LC_{50} values, the presumable harmless concentration was calculated as 53.46 ppm to which the fish were introduced. Haematological studies were carried out with reference to Hb, PCV, TEC, TLC, and other absolute values such as MCV, MCH and MCHC. During the exposure period, a reduction in haemoglobin content, packed cell volume and total erythrocyte count was observed. During recovery period, the fish overcame the stress induced by the toxicant by the continuous detoxification process, as a result of which the Hb, PCV and TEC increased and TLC decreased progressively to attain the normal level.

INTRODUCTION

Generally, aquatic organisms are susceptible to pollution effects by pesticides as well as by other pollutants. However, organisms try to adapt to these changes by changing their metabolic activities. But, at higher concentrations, these pollutants can cause damage to physiological system by affecting the organisms either at organ and cellular levels or even at molecular level, which in turn cause changes in the biochemical composition, and can be used to study the different protective mechanisms of the body to resists toxic substance and detoxification. The pesticides cause a number of subsidiary problems like affecting the ecosystems, and growth, reproduction and behaviour by causing pathological and physiological changes (Meenakshi 1993, Holden 1972, Anon 1962). Similarly, the fish when exposed to pesticides also show a shift in the metabolic pathway and change in glycogen mobilization in the tissues. Baskaran (1991) reported that the glycogen level decreased in fishes exposed to pollutant containing media.

Besides, the pesticides also bring some changes in the blood parameters. Thus metabolic status in an organism is very much reflected in its milieu interior (Lu 1985). Blood being the medium of intercellular and intracellular transport, comes in direct contact with various organs and tissues of the body, therefore, the physiological state of an animal at a particular time is reflected in its blood. Pesticides rapidly bind to the blood proteins and induce haematological changes such as changes in blood glucose, serum protein and serum cholesterol levels. These changes are of some value in assessing the impact of exposure under natural conditions and may also serve as tools for biological monitoring.

MATERIALS AND METHODS

Haematological Parameters

Collection of Blood: Fish were taken from the cement cisterns and the required amount of blood was obtained by caudal puncture of the fish using a heparinised syringe.

Estimation of haemoglobin content of blood (Hb): Haemoglobin content of blood was estimated using Sahli's haemometer (Superior, Germany) with permanent coloured glass comparison standards. The haemometer tube was filled to the level of lowest graduation (0.02g) with standard hydrochloric acid of dilution 1:10. Using the capillary pipette, 20 cu mm of blood was blown into hydrochloric acid, already present in haemometer tube. Blood was sucked into the pipette several times and blown out again. The haemometer tube was placed in the stand and the blood was diluted using distilled water with constant stirring until the colour matched with the colour of the standards. Reading was taken directly from the haemometer tube exactly three minutes after the blood was added to the acid. Haemoglobin content of blood was read in terms of grams of haemoglobin per 100 mL of blood.

Determination of packed cell volume (PCV): Blood was sucked into the heparinized haematocrit capillary tube (7.5 cm length, 0.1 cm width). After sealing both the sides of the tube it was centrifuged in the microhaematocrit centrifuge at 6000 rpm for 2 min. From the volume of blood taken and cell volume after centrifugation, the PCV percentage was calculated.

Erythrocyte count (RBC): The Neubauer's counting chamber was used for enumeration of the red blood cells. For counting the red blood cells the blood was diluted 200 times using Hayem's fluid (0.5g sodium chloride; 2.5 g sodium sulphate, 0.20g mercury perchloride and 100 mL distilled water). Sodium chloride and sodium sulphate together make the fluid isotonic with blood; sodium sulphate prevents clumping of cells and mercury perchloride acts as preservative and fixative. The number of red blood cells were counted after introducing this diluted blood into the haemocytometer and erythrocytes were represented in millions of cells/cu mm of blood. The RBCs of the control and treated fish were counted.

Leucocyte count: The Neubauer double counting chamber was used for white blood cell counting. The freshly shed blood was diluted 20 times using Turk's fluid (0.025g gentian violet, 3 mL glacial acetic acid and 97 mL distilled water). Gentian violet, stains leucocytes and glacial acetic acid destroys the red blood cells thus making the viewing of leucocytes easier. The diluted blood was fed into a haemocytometer and the leucocytes were counted under the microscope and were represented in number of cells/cu mm of blood. The leucocytes of the control and treated fish were counted.

Calculation of MCHb: The mean corpuscular haemoglobin content was calculated by using the values of haemoglobin content and the red blood cell counts and expressed in pg (Anderson & Klontz 1965).

Mean Corpuscular haemoglobin concentration (MCHC): The % of mean corpuscular haemoglobin content was calculated by using the values of haemoglobin content and the PCV% (Anderson & Klontz 1965).

Calculation of mean corpuscular volume (MCV): The mean corpuscular volume was calculated by using the values of PCV% and the red blood cell counts and expressed in μm^{-3} (Anderson & Klontz 1965).

RESULTS

Presumable Harmless Concentration (C)

In the present investigation, the presumable harmless concentration of monocrotophos to *Cyprinus carpio* was calculated as 51.57 ppm.

Haematological Parameters

There was significant alternation in the haematological value after 15 days of exposure to monocrotophos of sublethal concentration (Table 1). The control and exposed animals showed 13.01 $\pm$ 0.23 and 8.02 $\pm$ 0.01 percent of haemoglobin respectively. Decrease in the haemoglobin content was observed at the end of exposure period (Table 1). The haemoglobin content gradually increased to the level of 12.54 $\pm$ 0.31 after the recovery period of 15 days. The recovery phase of haemoglobin content is shown in Table 2. The amount of haemoglobin regained was found to be nearly 95% at the end of recovery period. The packed cell volume (PCV) and total erythrocytes count (TEC) decreased significantly during exposure period. During the recovery period, the packed cell volume and total erythrocyte count attained the value of 18.01 % and 1.43 millions per mm^3 respectively. The variations in PCV and TEC values during recovery period are shown in Table 2.

The total leucocyte count increased progressively when compared to control. In control fish, the leucocyte count was found to be $11.3 \times 10^3/mm^3$. After 15 days during the recovery period, the leucocyte count gradually decreased. In control recovery animals had the leucocyte count of $10.9 \times 10^3/mm^3$, whereas in pretreated fish it was $30.3 \times 10^3/mm^3$.

In other parameters like, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC), the absolute values also showed a decreasing trend under monocrotophos exposure period. During the exposure period of 15 days the MCH, MCHC and MCV values of control animal were 41.19 (pg), 31.81 (g/L) and 113 (μm^{-3}) respectively. But in monocrotophos exposed animals these parameters decreased considerably to 30.3 (pg) 29.43 (g/L) and 95.13 (μm^{-3}) respectively.

The fish exposed to sublethal concentration were transferred to monocrotophos impoverished water. The fish pretreated with sublethal concentration showed a slight increase in parameters when compared with exposure period. During recovery phase the control animals have MCH, MCHC and MCV, 39.18 (pg) 30.81 (g/L) and 110 (μm^{-3}) respectively while in the recovered animal, these values gradually increased to 35.23 (pg) 29.41 (g/L) and 95.13 (μm^{-3}) respectively (Tables 1, 2).

DISCUSSION

The data presented in Tables 1 and 2 clearly reveal that there are definite changes in the haematological parameters of *Cyprinus carpio* following exposure of monocrotophos for 15 days and another 15 days for recovery period. The haemoglobin content of the exposed fish exhibited a definite decrease as compared to normal fish upon exposure for 15 days to insecticide. The increased trend in reduction of the haemoglobin content was also observed. The present study was supported by the findings of Waluga (1966) who has recorded a reduction in haemoglobin content in bream *Abramis brama* exposed to sublethal concentration of phenol. A reduction in haemoglobin content was also noticed in the yearlings of *Coho salmon* upon exposure to pulp mill effluent (Mc Leay 1973). The reduction of haemoglobin in the present study might be attributed to the blood coagulation as suggested in bream *Abramis brama* (Waluga 1966) and *Cyprinus carpio* (Paul Raj 1982).

Table 1: Effect of sublethal concentration of monocrotophos on haematological parameters of *Cyprinus carpio* during the exposure period.

Blood Parameters	Control	Exposure period	Percentage of alteration
Hb (g%)	13.01 ± 0. 23	8.02 ± 0.01	-38.3
PCV (%)	34.01 ± 1.13	18.01 ± 0.02	-47.0
TEC× 10^6/mm^3	2.61 ± 0.12	1.43 ± 0.01	-45.2
TLC × 10^3/mm^3	11.3 ± 0.01	53.20 ± 0.05	-52.9
MCH (pg)	41.18 ± 2.11	30.3 ± 3.14	-26.4
MCHC (g/L)	31.81 ± 1.1	27.43 ± 0.06	-13.7
MCV (μm^{-3})	113 ± 1.31	85.13 ± 0.02	-24.6

Values are expressed as Mean + S.D. N = 5; % of alteration: (-) depletion and (+) elevation over control values.

Table 2: Effects of sublethal concentration of Monocrotophos on haematological parameters of *Cyprinus carpio* during recovery period.

Blood Parameters	Control	Recovery Period	Percentage Change
Hb (g%)	14.02 ± 0. 14	12. 54 ± 0.31	-10.50
PCV (%)	31.05 ± 0.13	24.02 ± 1.15	-22.60
TEC x 10^6/mm^3	2.43 ± 0.11	1.95 ± 0.13	-19.70
TLC x 10^3/mm^3	12.9 ± 0.01	15.34 ± 0.05	18.41
MCH (pg)	39.18 ± 2.10	35.23 ± 1.3	-10.08
MCHC (g/L)	30.81 ± 1.4	29.41 ± 0.03	-4.54
MCV (μm^{-3})	110 ± 1.43	95.13 ± 0.03	-13.50

Values are expressed as Mean + S.D. N = 5; % of alteration: (-) depletion and (+) elevation over control values.

Germysz-Kathowaska et al. (1985) reported a decline in haemoglobin, haematocrit (PCV) and RBC count by an organophosphorus insecticide in Japanese quail. This reduction can be related to the decreased RBC number which indicates haemolysis, haemorrhage and reduced erythropoiesis in fishes on exposure to insecticide.

The low Hb concentration appears to be related to its obligatory air breathing habit and due to low oxygen content in the water where the fish reside. The reduction in RBC count indicates anaemia associated with erythropenia which is similar to earlier reports of Panigrahi et al. (1978) and Agarwal et al. (1983). The anaemia with erythropenia has also been reported earlier in fishes after exposure to sublethal doses of mercury, lead and Rogor (Srivastava & Mishra 1979, Goel & Maya 1986).

Leucocytosis is evidenced by the increase of WBC count. Leucocytosis has been considered to be an adaptation of animals to meet stressful conditions. WBC count also depleted along with Hb. RBC count indicates dysfunctioning of haemopoietic system along with dysleucopoeisis. This is most probably due to bone marrow depression and liver dysfunction. It can also be said that during exposure of monocrotophos the extent of toxic effect in fishes increase in a particular manner.

TLC of *Cyprinus carpio* exposed to monocrotophos showed a significant increase. Increase in TLC in the test fish could be due to lymphopoiesis and/or enhanced release of lymphocytes from lymphomyeloid tissues. The increase in lymphocyte number in treated fish is also probably for the removal of cellular debris of necrosed tissue at a quicker rate as reported by Mc Leay (1974). The

increase in leucoytes may be due to interference of monocrotophos toxicity in the development of lymphocytes in haemopoietic tissues leading to leukaemogenic condition or the immunological response to meet the stress caused by monocrotophos toxicity. The above finding were supported by Saxena & Parashari (1983). Further, the increase in WBC count could be attributed as an adaptive value to fishes under stress. In fact, Henry et al. (1978) suggested that the immunological mechanism could stimulate leucocytosis in response to stresses.

In monocrotophos exposed fish, the decrease in TEC was observed after exposure period, but there was a slight rise after completion of recovery period though the normal number was not achieved. This increase in TEC suggests compensatory erythropoiesis due to the stimulatory effects. The increase observed was because of increase in immature erythrocyte count (Patil & Dhande 2000).

A significant decrease was observed in total leucocyte count of *Channa punctatus* after exposing the fish to ammonia in sublethal concentrations, i.e. half and two-third of LC_{50} for 28 days (Ravindar Kumar & Anand Muni 1997). Dabrowaska & Wlasow (1986) have also observed decrease in leucocyte counts in *Cyprinus carpio.* The development of leucopenia in the catfish could be due to aldrin interference to haemopoiesis (Bansal et al. 1979). Leucopenia has specific response of fish to stress and toxicants (Hickely 1976).

The present study reveals that haemoglobin percentage, packed cell volume and total erythrocyte count decreased significantly while total leucocyte count increased progressively in sublethal concentrations. Other absolute values such as MCV, MCH and MCHC also altered in response to the changes in above parameters. Similar finding of haematological changes was made in common Indian catfish *Heteropneutes fossilis* under nickel stress (Prasanta Nanda 1997).

Insecticides are known to alter the blood parameters of fishes. A significant decrease in RBC, Hb content and PCV has been observed earlier in fishes exposed to different pesticides (Koundinya & Ramamurthy 1979, Sambasiva Rao et al. 1955) and such decreasing effect has been primarily attributed to a condition of hypochronic microcytic anaemia (Bhai et al. 1971, Raja Rishi 1986). The finding of present investigation is also similar to such decreasing trends in some blood parameters such as Hb, PCV, MCH and MCV suggesting the haematological toxicity of monocrotophos. A significant increasing trend, observed in the number of WBC, has been reported by Sekar & Christy (1996) in catfish exposed to sublethal concentration of phosphamidon. MCV and MCH along with MCHC showed appreciable decrease in exposed fishes. The decreased MCV, MCH and MCHC clearly indicate the hypochronic microcytic anaemia. Ramalingam et al. (2000) reported that decrease in MCV, MCH and MCHC in freshwater fish *Cirrhina mrigala* when exposed to lead (acute exposure). So, it can be concluded that present investigation strongly suggest a possible alteration in the fish as a whole.

During the recovery period, the fish overcame the stress induced by the toxicant by continuous detoxification process, and as a result of which the Hb, PCV and TEC increased and TLC decreased to the normal level.

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