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Original Research Paper

Acute Toxic Effects of Parathion on Leucocytes of an Air Breathing Fish *Clarias batrachus*

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ABSTRACT

The present investigation deals with the toxic effect of a common insecticide parathion on total and differential count of an air-breathing fish *Clarias batrachus*. In four different concentrations (2 ppm, 4 ppm, 6 ppm and 8 ppm, of parathion for 96 hrs to *Clarias batrachus*, a gradual and significant increase in total leucocytes with parathion concentrations was observed. Total percentage of lymphocytes showed marked increase in all the test animals exposed to different concentrations of the toxicant. Significant decrease in neutrophils, basophils, eosinophils and monocytes were also observed in parathion exposed animals.

INTRODUCTION

Normal water conditions are necessary for proper fish culture. Pesticides, once used indiscriminately, alter physico-chemical properties of water and make the fish life difficult. Parathion (an organophosphorus insecticide) has been found to be highly toxic not only to human and insects but also to fishes and to those animals, which constitute the food of fishes. It is used against a wide range of insects and mites on crops.

The haematological parameters are considered as diagnostic indices of pathological conditions in animals. Such studies have been made in lower vertebrates taking into consideration the various environmental factors (Kristofferson et al. 1974). Blood is the primary target of pesticidal action (Kennedy et al. 1970). Alterations in haematological parameters due to water pollutants in poikilothermic animals particularly in fishes have been well established in recent years (Joshi 1982, Thakur 1992, Kumari et al. 2006, Paul et al. 2007, Sah et al. 2006, Tilak et al. 2007). But the studies on toxic effects of parathion in fishes, in general, and in air breathing fish *Clarias batrachus* in particular are meagre (Yadav & Paul 2007). Leucocytes are one of the important constituents of blood of vertebrates which are considered frequently for toxicological studies. Attempts have also been made to measure the impact of various toxicants on total and differential leucocyte count of several fishes in recent years (McLeay 1973, Nawman & McLean 1974, Ellis et al. 1978, Storozhuk & Guleva 1983, Rai et al. 1985, Thakur & Pandey 1990). The aim of the present study was to analyse the effect of commonly used insecticide parathion on total and differential leucocyte count of a common air breathing fish *Clarias batrachus*.

MATERIALS AND METHODS

In the present study, the common air breathing fish *Clarias batrachus* (both male and female) were used as experimental animals. Fish were procured from the local fish market and acclimatized at laboratory conditions for a fortnight with proper supply of fish food. Parathion was used as chemical

stressor. The experiments were designed for four different concentrations of parathion i.e., 2 ppm, 4 ppm, 6 ppm and 8 ppm for 96 hrs with control taking 10 fish of almost same weight (male & female separately) in each group.

A clean dry plastic syringe containing EDTA was used to take out blood from the cauda dorsalis of the fish. The total and differential counting of leucocytes was made with the help of Thoma-Zeiss haemocytometer with improved Neubauer ruling as described by Darmady & Davenport (1954).

RESULTS

The total count and differential count of leucocytes in male fish of *Clarias batrachus* were more than the female fish. The mean values of total and differential leucocyte count of experimental male and female fish are shown in Table 1 and 2 respectively and are presented in Figs. 1-6.

Total leucocytes: The range of leucocytes in control group of male fish was between 216.48 × $10^3/mm^3$ and 301.10 × $10^3/mm^3$, whereas the mean count was 243.509 ± 8.44 × $10^3/mm^3$. In control group of female fish, the range of leucocytes was from 201.26 × $10^3/mm^3$ to 305.07 × $10^3/mm^3$, whereas the mean count was 238.48 ± 10.1 × $10/mm^3$. The average number of total leucocyte count in 2 ppm, 4 ppm, 6 ppm and 8 ppm parathion intoxicated male and female fish showed increasing trend up to 6 ppm of parathion from the control values and after that there was decline in the leucocyte count (Fig. 1).

Lymphocytes: In control group of male fish minimum and maximum percentages of lymphocytes were 46% and 51% respectively, whereas the mean percentage of lymphocytes was 48.3 ± 0.6%. An increasing trend in the average percentage of lymphocytes in 2 ppm, 4 ppm, 6 ppm and 8 ppm parathion intoxicated male fish was found (Table 1, Fig. 2). In control group of female fish the range of lymphocytes percentage varied 44% to 49% with mean percentage of 46.3 ± 0.5%. Similar increasing trend in the mean lymphocyte percentage in 2 ppm, 4 ppm, 6 ppm and 8 ppm parathion intoxicated female fish was observed (Table 2, Fig. 2).

Neutrophils: The percentage of neutrophils in control group of male fish was between 25% and 28% having the mean count of 26.5 ± 0.38%. The mean percentage of neutrophils in 2 ppm, 4 ppm, 6 ppm and 8 ppm parathion intoxicated male fish showed decreasing trend (Table 1, Fig. 3). In control group of female fish, the neutrophil percentage ranged from 25% to 29% with decreasing trend in 2 ppm, 4 ppm, 6 ppm and 8 ppm parathion intoxicated female fish.

Monocytes: The range of percentage of monocytes in control group of male fish was between 7% and 22%, whereas the mean percentage was 14.9 ± 1.64%. In control group of female fish, the range of percentage of monocytes was 125 to 20% with mean percentage of 15.8 ± 2.16%. A decreasing trend in the mean percentage of monocytes in 2 ppm, 4 ppm, 6 ppm and 8 ppm of parathion intoxicated in male and female groups of *Clarias batrachus* was observed (Tables 1, 2 and Fig. 4).

Basophils: The range of percentage of basophils in control group of male fish was found to be 4% to 8%, whereas the mean percentage was found to be 5.9 ± 0.41.%. The average percentage of basophils in 2 ppm, 4 ppm, 6 ppm and 8 ppm parathion intoxicated male fish showed decreasing trend (Table 1, Fig. 5). In control group of female fish, the minimum and maximum percentage of basophils were 5% to 9%, whereas the mean percentage was 6.6 ± 0.49%. Similar trend was also observed in the mean percentage of basophils in 2 ppm, 4 ppm, 6 ppm and 8 ppm parathion intoxicated female fish (Table 2, Fig. 5).

Eosinophils: The range of percentage of eosinophils in control group of male fish was between 3%

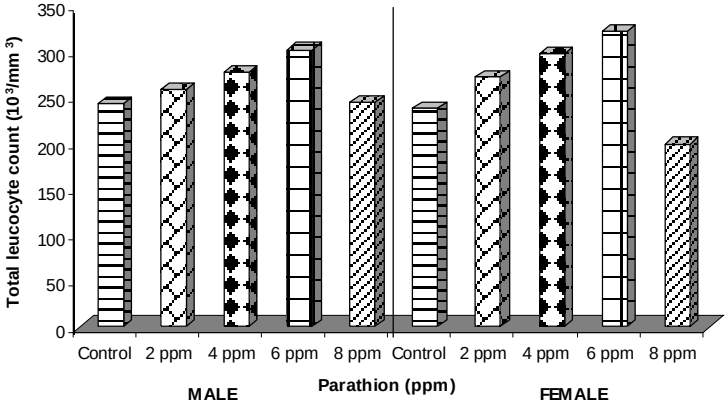

Fig. 1: Effect of parathon toxicity on total leucocyte count of *Clarias batrachus.*

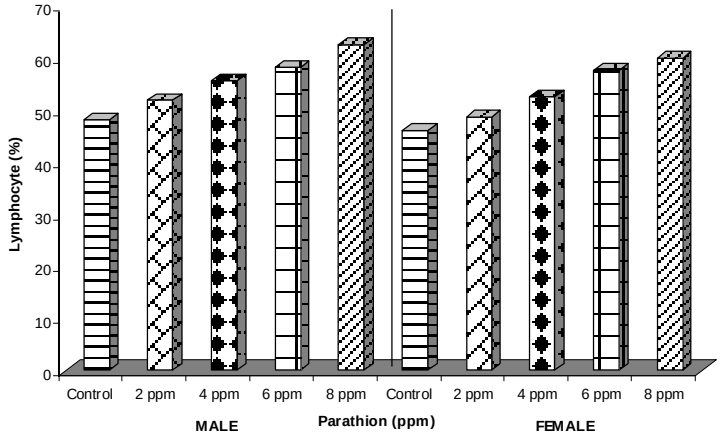

Fig. 2: Effect of parathon toxicity on lymphocyte (%) of *Clarias batrachus.*

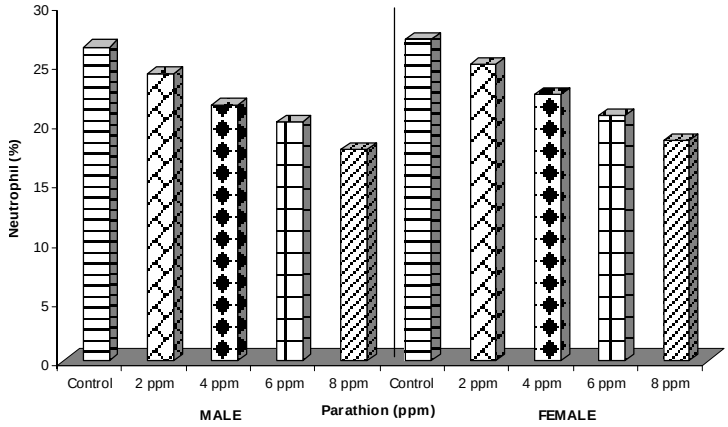

Fig. 3: Effect of parathon toxicity on neutrophil (%) of *Clarias batrachus.*

and 7% with the mean percentage of eosinophils of 4.7 ± 0.44% (Table 1, Fig. 6). In control group of female fish the range of percentage of eosinophils was between 1% and 6%, whereas the mean percentage was 3.6 ± 0.55%. In case of eosinophil percentage also, there was a decreasing trend when both male and female fish of *Clarias batrachus* were treated with different increasing concentrations of parathion.

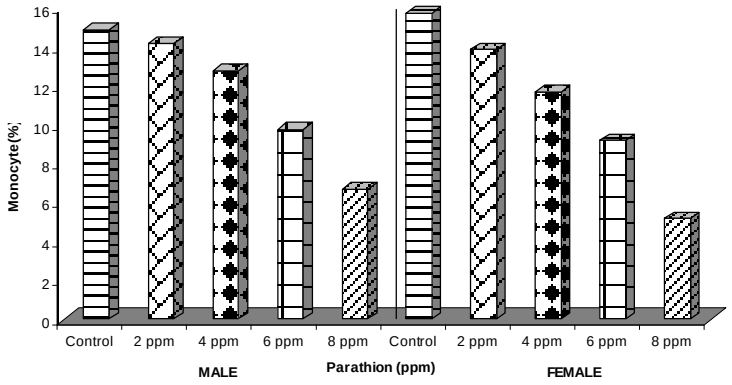

Fig. 4: Effect of parathon toxicity on monocyte (%) of *Clarias batrachus.*

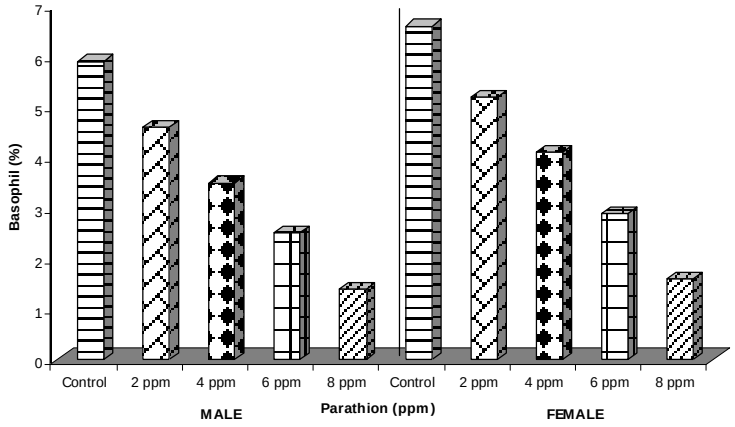

Fig. 5: Effect of parathon toxicity on basophil (%) of *Clarias batrachus.*

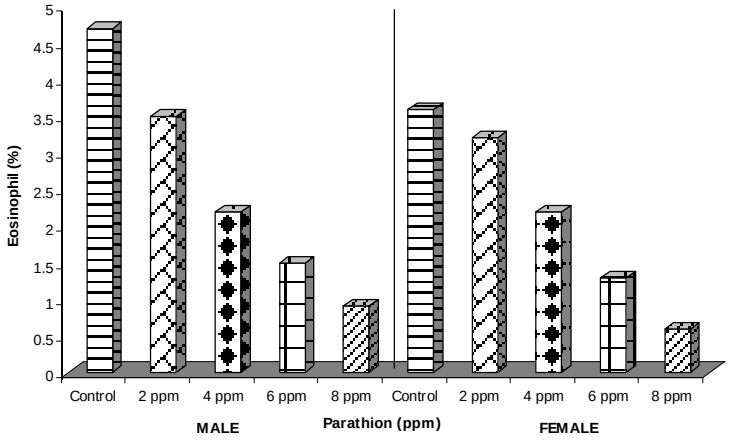

Fig. 6: Effect of parathon toxicity on eosinophil of *Clarias batrachus.*

Table 1: The effect of parathion toxicity on total and differential count of leucocyte of male *Clarias batrachus.*

Sl.No.	Parameters	Control	Parathion concentration (ppm)			
			2.0	4.0	6.0	8.0
1.	Leucocyte(10^3/mm^3)	243.509±8.45	258.07±10.81	277.86±11.29	302.53±10.83	245.58±13.41
2.	Lymphocyte (%)	48.3±0.6	52.0±0.67	55.8±0.52	58.4±0.53	62.7±0.56
3.	Neutrophils (%)	26.5±0.38	24.2±0.57	21.6±0.53	20.1±0.59	17.8±0.56
4.	Monocytes (%)	14.9±1.64	14.2±1.41	12.8±1.25	9.8±0.94	6.7±0.84
5.	Basophils (%)	5.9±0.41	4.6±0.4	3.5±0.38	2.5±0.34	1.4±0.28
6.	Eosinophils (%)	4.7±0.44	3.5±0.48	2.2±0.44	1.5±0.35	0.9±0.22

Table 2: The effect of parathion toxicity on total and differential count of leucocyte of female *Clarias batrachus.*

Sl.No.	Parameters	Control	Parathion Concentration (ppm)			
			2.0	4.0	6.0	8.0
1.	Leucocyte(10^3/mm^3)	238.48±10.01	272.49±1.34	297.51±4.76	321.69±6.99	199.90±9.60
2.	Lymphocyte (%)	46.3±0.5	48.8±0.67	52.6±0.56	57.7±0.42	60.10±0.57
3.	Neutrophils (%)	27.1±0.45	25.0±0.52	22.5±0.63	20.7±0.61	18.6±0.51
4.	Monocytes (%)	15.8±2.16	13.9±1.0	11.7±0.09	9.2±0.57	5.2±0.39
5.	Basophils (%)	6.6±0.49	5.2±0.52	4.1±0.41	2.9±0.41	1.6±0.35
6.	Eosinophils (%)	3.6±0.55	3.2±0.50	2.2±0.34	1.3±0.24	0.6±0.15

DISCUSSION

The knowledge of haematological indices makes it possible to learn more objectively the condition and function of fishes and their response to external influences. Blood often exhibits pathological changes before the appearance of any external symptoms of toxicity. Therefore, the haematological studies in animals form a promising tool for the investigation of physiological alterations caused by environmental pollutants. Blood, being the medium of intercellular and intracellular transport, comes in direct contact with various organs and tissues of the body, the physiological state of an animal at a particular time is reflected in its blood.

The total leucocytes count showed gradual increase in their number from 2 ppm parathion concentration up to 6 ppm parathion concentration but decreased at 8 ppm. An increase in the number of leucocytes may be to cope up with the removal of cell debris of necrosed tissues under the toxic stress (Goel et al. 1984). A fall in the total count of WBC after 6 ppm of parathion concentration appears to be because of a fall in the percentage count of neutrophils and other leucocytes excepting lymphocytes during prolonged period of chemical stress. Neutropaenia and monopaenia, as recorded in the present investigation in *Clarias batrachus* exposed to parathion, might have caused leucopaenia. It appears that neutropaenia results into leucopaenia as in the case of mammals. Such type of observations in total leucocytes have also been reported in *Channa punctatus* by Garg et al. (1982). Shammi & Qayyum (1982) reported a significant change in differential count of leucocytes after administration of 5.50 ppm and 10.0 ppm of carbyl concentrations in *Clarias batrachus.* It was observed that lymphocyte percentage had significantly been increased causing lymphocytosis in animals exposed to various parathion concentrations. Sharma & Gupta (1982) found an increase in lymphocytes, and a fall in the basophils and monocytes count in *Clarias batrachus* exposed to carbon tetrachloride. *Channa punctatus* after 2',4'-diamine-3'-aminoazo benzene treatment showed increase in large lymphocytes and neutrophils, and decrease in thrombocyte counts (Garg et al. 1982). Increase in

neutrophils has also been reported in Coho Salmon exposed to industrial effluents (Mcleay 1973). Rai et al. (1985) reported decrease in the percentage of monocytes, large lymphocytes and neutrophils, and increase in number of thrombocytes. Lymphocytosis as evidenced in present investigation might be due to immunological reaction to produce more antibodies to cope up with the stress induced by the toxicant.

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