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Effect of Some Pesticides on Leucocytes of a Fresh Water Fish *Channa gachua* (Ham.)

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Abstract

The study deals with the toxic effects of some commonly used pesticides (lindane, pyrethroid and dhanusan—50) on the total and differential leucocyte counts of a fresh water fish, *Channa gachua* (Ham). A significant variation in total and differential leucocyte counts in the test fish exposed to three pesticides in LC_{50} doses for 24, 48, and 96 h duration was recorded showing leucopenia. There was a rise in neutrophil, basophil, eosinophil and monocyte counts whereas a fall in lymphocytes was observed. Probable reasons for these changes were specific responses to stress caused by the toxicants and probably the toxins might have increased phagocytic components to fight against the adverse conditions.
Key words : Pesticides, Leucocytes, Fresh water fish, *Channa gachua.*

Water being essence of life is polluted with various synthetic chemicals coming through industrial effluents, domestic sewage and agricultural run-off. Pesticides are the common example of such chemicals used extensively in the agricultural to boost up crop production. These chemicals reach the water bodies through different routes and pose potential health hazards to the aquatic biota in general and fishes in particular. The blood is taken as an important tool to assess the health condition of an organism. As such, any change in the blood composition due to pesticide poisoning will be of immense help to arrive at correct assessment of toxicity of pesticides (1). Alterations in hematological parameters due to water pollutants in the poikilothermal animals specially fishes have been well established (2—9). Leucocytes are due of the important constituents of blood which are considered frequently for toxicological studies (10—15). In the present investigation, an attempt was taken to evaluate the toxic effects of some pesticides on the leukocyte counts of a fresh water fish, *Channa gachua* (Ham.).

Methods

Live, healthy specimens of the fish, Channa gachana (Ham.) were collected from local fresh water ponds and were brought to the laboratory in polythene bags containing pond water. They were washed with 0.1% $KMnO_4$ solution to avoid dermal infection. In the laboratory the fish were kept in large aquaria to avoid overcrowding and were allowed acclimatization for 15 days with provision of fish food. The quality of the test aquaria was maintained following recommendation of APHA et al. (16). The pesticides selected for the present hematological study were, lindane (organochlorine), pyrethyroid (cypermethrin) and dhanusan-50 (organophosphate pesticide).

For each pesticide three sets of aquaria were taken and each contained pre determined LC_{50} dose of the test chemical in varied exposure period. Ten acclimatized fish were kept in each aquarium containing LC_{50} dose for 24, 48 and 96 h of treatment with the respective pesticides (lindane, pyrethroid and dhanusan). A control experiment with 10 fish was set simultaneously for the same duration without toxicant for comparison with data obtained after conducting the hemotoxicity test with various concentrations of the three different pesticides.

At the termination of each exposure period fish both from control and experimental aquaria were taken out, sacrificed and blood was collected from the caudal peduncle by a heparinized syringe. The total and differential counting of the leucocytes was made with the help of Thomas-Zeiss hemocytometer with im-

Table 1. Alterations in total leucocyte counts of *Channa gachua* at LC_{50} dose of some pesticides (value in $10^3/mm^3$). ± = Standard deviation.

Pesticides	Control	LC_{50}/24 hr. dose	LC_{50} 48 hr. dose	LC_{50} 96 hr. dose
Lindane	25.10±0.50	27.4±0.60	21.2±0.01	19.10±0.70
Pyrethroid	25.30±0.40	28.10±1.10	21.0±0.35	20.20±0.56
Dhanusan	25.20±0.40	28.0±0.35	22.2±0.33	21.75±0.54

proved neubauer ruling (17).

Results and Discussion

Changes in total and differential leucocyte counts in the test fish, *Channa gachua* exposed to three pesticides in LC_{50} doses under 24, 48 and 96 h duration in the present study, have been summarily given in Tables 1—4. The total leucocyte count (TLC) in the control fish, which were kept in normal water and sampled with each experiment ranged between 25.10 × $10^3/mm^3$ and 25.3 × $10^3/mm^3$. The TLC in the fish exposed to lindane in LC_{50} concentration for 24, 48 and 96 h increased initially but later on got decreased, creating a leucopenic condition in the fish. The TLC value of the test fish under LC_{50}/24 h intoxication raised to 27.4 × $10^3/mm^3$ showing an increase of 9.16% from the control value but the total leucocyte count in fishes exposed to LC_{50}/48 h period exhibited a gradual fall, the value being 21.2 × $10^3/mm^3$ showing a decrease of 15% from the control. Similarly the value also had a sharp fall in the fish exposed to LC_{50}/96 h dose, it being 19.1 × $10^3/mm^3$ with a decrease of 24% over the control value (25.1 × $10^3/mm^3$).

In the fish exposed to three concentrations of pyrethroid, the total leucocyte count exhibited an increase at 24 h exposure, the value being 28.10 × $10^3/mm^3$ showing an increase of 11% over the control value. However, a gradual decrease in the TLC value was recorded in the fish exposed to LC_{50} dose for 48 hours and 96 h. The TLC value in these cases were 21.0 × $10^3/mm^3$ and 20.20 × $10^3/mm^3$ respectively which were respectively 17 and 20% less than control value.

In the fish exposed to Dhanusan a similar trend of rise and fall in TLC was observed. In 24 h exposure to the LC_{50} concentration of the pesticide, the TLC value was 28.0 × $10^3/mm^3$ showing an increase of 11% from the control value. But in fish under 48 h exposure period in the LC_{50} dose, the total leucocyte count had a downward trend, the same being 22.2 × $10^3/mm^3$ which was 12% less than the control value. A further fall of 14% in the TLC value was recorded in fish exposed to LC_{50} dose for 96 h duration over the control value (Table 1). It was evident that higher concentration of the pesticides for longer exposure period caused leucopenia in fishes.

Table 2. Changes in differential count of leucocytes of *Channa gachua* after intoxication with lindane (valves in percentage). ± = Standard deviation.

Leucocyte types	Control	24 hr. LC_{50} dose	48 hr. LC_{50} dose	96 hr. LC_{50} dose
1 Large lymphocyte	29.7±0.5	32.0±0.62	31.6±0.30	33.9±1.10
2 Small iymphocyte	54.5±0.90	52.3±0.45	53.5±0.40	48.8±1.20
3 Monocyte	5.9±0.30	6.3±0.25	6.10±0.35	7.8±0.60
4 Neutrophil	3.8±0.35	3.2±0.35	3.3±0.25	3.1±0.20
5 Eosinophils	3.6±0.62	3.7±0.20	3.8±0.65	3.9±0.15
6 Basophils	2.5±0.55	2.6±0.70	1.7±0.15	2.5±0.50

The differential count of leucocytes in the fish exhibited the fall of WBC and their percent range in the control as follows : Large lymphocytes 27.3 to 28.6%, small lymphocytes 54.5 to 57.7%, Monocytes 4.7% to 6.3%, Neutrophils 1.6—3.8%, Eosinophils 3.6—5.3%, Basophils 2.0—3.2%.

In lindane toxicity, it was observed that large lymphocytes increased in all exposure period, the maximum increase of 14% was recorded in the fishes exposed for 96 h in LC_{50} dose. On the other hand, the small lymphocytes exhibited a gradual decrease in three cases. In this case also a marked fall of about 10% was recorded in the fishes exposed to LC_{50} concentration for 96 hours of exposure. However, monocytes exhibited a gradual increase under 24, 48 and 96 h intoxication in LC_{50} doses. Rest of the components like neutrophils eosinophils and basophiles showed a minor variation (Table 2).

In fish exposed to three concentration of pyrethroid for 24, 48 and 96 h period the differential leucocyte counts also under went variation in its percentage. There was a moderate increase in the percentage

Table 3. Changes in differential leucocyte count of *Channa gachua* after treatment with pyrethroid (valves in percentage). ± = Standard deviation.

Leucocyte types	Control	24 h LC_{50} dose	48 h LC_{50} dose	96 h LC_{50} dose
1 Leucocyte types	27.30±1.3	33.9±1.20	31.5±0.4	33.3±1.10
2 Small iymphocyte	57.70±0.35	47.5±1.10	48.9±0.50	45.0±1.30
3 Monocyte	6.30±0.70	7.7±0.60	7.9±0.33	7.80±0.45
4 Neutrophil	1.80±0.10	2.40±0.25	2.85±0.30	3.20±0.20
5 Eosino-phils	4.9±0.62	6.20±0.15	5.50±0.2	6.6±0.60
6 Basophils	2.0±0.50	2.30±0.50	3.35±0.55	3.10±0.30

Table 4. Changes in differential leucocyte count of *Channa gachua* after treatment with pyrethroid (valves in percentage). ± = Standard deviation.

Leucocyte types	Control	24 h LC_{50} dose	48 h LC_{50} dose	96 h LC_{50} dose
1 Leucocyte lymphocyte	28.6±1.10	35.6±1.15	31.7±0.80	29.6±1.30
2 Small iymphocyte	56.7±1.20	48.1±1.10	52.0±0.35	54.5±1.15
3 Monocyte	4.7±0.30	5.41±0.45	5.7±0.33	7.80±0.45
4 Neutrophil	1.6±0.62	2.7±0.20	3.2±0.28	2.6±0.30
5 Eosinophils	5.3±0.20	5.8±0.60	5.4±0.50	5.6±0.55
6 Basophils	3.2±0.30	2.2±0.22	2.7±0.15	2.3±0.45

of lage lymphocytes, the maximum increase of 25% was found in 96 hours intoxication period. Similar increase was observed in monocytes, neutrophils, eosinophils and basophils as given in Table 3. The small lymphocytes had a gradual fall in their number, the maximim fall in percentage was found at 96 hours of intoxication period.

A similar pattern of variation in different types of leucocytes was observed in fishes exposed to LC_{50} dose of dhanusan for 24, 48 and 96 h period. There was a maximum increase of 24% in large lymphocytes under 24 h in toxication period but for 48 and 96 h duration the lymphocytes showed a decrease in their percentage. The small lymphocytes also had a fall in their number in all the three exposure periods whereas the monocytes in the fish of all the three groups had a moderate increase in number. Similarly, the neutrophils also exhibited a gradual and significant increase in their percentage in all exposure periods. The eosinophils in the test fish had a marginal increase in all the three exposures whereas a marginal fall in the percentage of basophil was recorded in fish exposed to three concentrations of dhanusan (Table 4).

The leucocytes in fish play the same role as that of other vertebrates. The leucocyte number falls when the animal comes in contact with some chemicals. In the persent study a leucopenic condition was observed in fishes exposed to LC_{50} dose for 24, 48 and 96 h of treatment with lindane, pyrethroid (cypermethrin) and dhanusan. The percentage of decrease in total leucocyte count was more in lindane exposure, followed by phrethroid and dhanusan.

Leucopenia, an overall reduction in the leucocytes, has been demonstrated in teleosts exposed to pesticides and metals (18—20). Mount and Putnick (21) reported leucopenia in fishes due to endrin treatment and believed this to be the cause for mass killing of fish in river Mississippi. A similar result was observed by Mahajan and Juneja (22) in *C. punctatus* due to aldrin intoxication. Among other workers who have reported leucopenia in fishes under exposure to toxicauts (23—25).

The depletion in the total leucocyte count due to pesticide toxicity in fishes has been attributed to many factors by several workers. Hickey (26) was of the view that leucopenia occurs due to specific responses to the stress caused by the toxicants, while Srivastava and Agarwal (27) were of the view that increase in the circulatory level of corticosteroid causes leucopenia in fishes. However, Tovassoli (28) reported that pesticides affect nervous system and since neural elements are associated both with blood vessel and stroma, it affected the haemopoiesis resulting in lencopenic condition in fishes exposed to pesticides. According to Wright (29) decease in total lencocyte count suggests that the fish is losing its capacity to defend microbial or bacterial infection and autolysis caused by the activity of certain hydrolytic enzymes like phosphatase, lipase releaed in to the blood stream due to pesticide stress. In the present investigation decrease in total leucoeyte count in the fish exposed to the pesticides due to depression of lencopoiesis or disintegration of WBC because leucocytes combat any toxicant introduced into the blood stream. Reduction in the number of circulatory leucocytes may also be due to increased level of circulating ACTH

and corticosteroid hormdue. The fall in total count of WBC in the present study may also be due to interference in the nervous system by the toxic effects of lindane and pyrethroid pesticides because the two are known as neurotoxic compounds. More over regarding the action of dhanusan causing lencopenia in the test fish, it is well established that dhanusan inhibits Ach E causiny disturbance in oxygen consumption by tissues. This ultimafely creates stressful condition in fishes and probably this may be the cause of reduction in the lencoeytes in fishes in the present study.

The differential count of leucocytes in the present study exhibited a markedvariation due to different exposures of lindane, pyrethroid and dhanusan. There was a significant increase in large lymphocytes whereas small lymphocytes showed a decrease in all the cases. Such studies have also been made by other workers in the past. Srivastava and Agarwal (27) reported significant decrease in small lymphocytes due to sublethal dose of cobalt in *Colisa fasciatus* for 15 days. A similar result was reported by Singh (30) in *Anabas testudineus* due to exposure to rogor. Pathak (31) also observed lymphocytosis in *H. fossilis* due to simizin. It was observed that lymphocyte percentage had significantly increased causing lymphocytosis in animals exposed to various parathion concentrations (14). Rai et al. (10) reported decrease in the percentage of monocytes, large lymphocytes and neutrophils and increase in the thrombocytes. In the present study a fluctuating trend was observed in neutrophils and basophils in higher concentration of lindane, dhanusan and pyrethroid. No specific reason has been assigned by the previous workers although they were of the view that stress caused by the pesticide poisoning may be one of the factors responsible for there alterations. In the present investigation it is opined that lymphocytosis might be due to immunological reactions to produce more antibodies to cope up with the stress caused due the toxicants. The nervous and hormonal impairment due to toxic effects of the pesticides might account for change in differential leucocyte counts due to lindane, pyrethroid and dhanusan.

Thus, it is concluded that the pesticides induce alterations in the hemostatic mechanism of fishes and whether any compensatory mechanism is involved to maintain the same in fishes exposed to toxic effects of pesticides, it requires further elaboration.

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