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Effect of lindane on haematological indices of minor carp *Labeo boga* (Ham.)

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ABSTRACT

In the present study an attempt has been made to study the effect of lindane on haematological indices of minor carp *Labeo boga*. LC_{50} value of lindane after 96hrs. was found to be 3.5mg/l for *Labeo boga*. Examination of haematological indices was performed on both control and experimental specimens after exposure to lindane at varying concentrations ie. 0.35mg/l, 0.70mg/l and 1.05mg/l lindane (10%, 20% and 30% respectively of LC_{50} value of lindane. Total erythrocyte count (TEC), Haemoglobin (Hb), Haematocrit, (Hct), Mean corpuscular volume (MCV), Mean corpuscular haemoglobin concentration (MCHC), Mean corpuscular haemoglobin (MCH) and Total leucocyte count (TLC) has been found to exhibit varied results under various concentrations at different time periods.

Key words: Lindane, haematological indices, minor carp *Labeo boga*.

INTRODUCTION

Survival of life forms is threatened by the indiscriminate chemical pollution and other abuses of the natural resources of the earth. Run off of various harmful chemicals from the water extensive industrial units (like chemical, textile, dyeing, leather, pulp processing etc.) extend their toxic effect to all the aquatic organisms. The prevalence of these chemical pollutants has become a matter of great concern, as they are not processed/ degraded in natural conditions with in a reasonable time which after finding their entry into the aquatic ecosystems are taken up into the body of all aquatic organisms including fish. Although fish like any other organism tend to maintain their internal milieu to near normal by variety of regulatory adjustments but the problem begins when the level of these pollutants exceeds the threshold limits and effects increasingly became deleterious at the cellular level. To determine the extent of damage in the exposed fish in terms of structure and function, it is important to evaluate different variables that are affected by the toxicants viz., feeding, respiration, osmoregulation, reproduction including alteration in blood composition.

Fish culturists and biologists have long considered the application of haematological techniques for the diagnosis, prevention and treatment of fish diseases.

Analysis of haematological studies (Lu 1985, Tort and Torres 1988, Narayan and Singh 1991, Christensen *et al* 1992, Rodriguez 1995, Hart *et al* 1997, Das and Mukherjee 2000 a & b, Sarkar *et al* 2005, Rehman 2006, Velmurugan *et al* 2007, Verma 2007, Sachar (2008) help not only to identify the target organs of the toxicants but also influence or impairs the general health status of the fish besides providing an early warning signal inn stressed organs (Folmar, 1993).

Looking into the existing scenario, the present work encompasses the study on effect on lindane, an insecticide on the haematological indices in a minor carp, *Labeo boga* (Ham.), a fish commonly consumed by the locals.

MATERIAL AND METHODS

Adult specimens collected with the cast net from Nagrota stream of River Tawi, brought live to the laboratory and acclimatized for two weeks prior to experimentation. The 96h LC_{50} value of lindane was determined as 3.5 mg/l. Three sublethal concentrations i.e. 0.35mg/l, 0.70mg/l and 1.05mg/l lindane (10%, 20% and 30% respectively of LC_{50} value of lindane) were employed for the experiment. 0.5 ml of blood was taken directly by cardiac puncture with the help of heparinized syringes containing EDTA as anticoagulant.

Among blood parameters TEC and TLC were counted with the help of improved Neubauer cytometer (Shaw, 1930). Hb% was estimated by using sahli's haemoglobinometer (Dethloff *et al*, 1999), Hct was determined by centrifugation method (Wintrobe, 1967). MCH, MCV and MCHC were calculated by the formulae.,

MCV in femtolitres=Hct × 10/ RBC/ RBC count

MCH in pg = MCH × Hb/RBC count

MCHC in % = Hb × 100/Hct

Statistical differences of haematological parameters between days were determined using one way ANOVA. Blood smears were scanned and photographed with Nikon Semi Digital Camera Microscope.

RESULTS AND DISCUSSION

Erythrocytes

Total eythrocyte count

Compared to control groups significant decline ($P< 0.99$) has been observed in TEC (fig.1) of the fish in all the treated groups (Table 4). Decline appears to be highest during the first half (1-15days) compared to the second half (15-30 days) of the

Table 1: Percental decline of Total erythrocyte count (TEC) of *Labeo boga* exposed to different sublethal concentrations of Lindane

Exposure period	Different Sublethal Concentrations Set I (10%)	Set II (20%)	Set III (30%)
1-5 Days	6.12%	7.5%	13.36%
5-10 Days	5.21%	8.55%	15.42%
10-15 Days	5.96%	8.86%	23.52%
15-20 Days	2.92%	2.70%	11.53%
20-25 Days	2.51%	2.77%	4.34%
25-30 Days	2.06%	6.28%	7.27%

Table 2: Percental decline of Haemoglobin (Hb%) of *Labeo boga* exposed to different sublethal concentrations of Lindane

Exposure period	Different Sublethal Concentrations Set I (10%)	Set II (20%)	Set III (30%)
1-5 Days	0%	3.22%	3.22%
5-10 Days	4.83%	6.66%	8.33%
10-15 Days	5.08%	5.35%	9.09%
15-20 Days	1.78%	1.88%	0%
20-25 Days	0%	0%	4%
25-30 Days	1.81%	3.84%	2.08%

experiment. Also the percental decline during 1-5 days (Table 1) is lesser in Set-I contrast to Set-2, where it is slightly higher. and Set-3, where it is more than double than that observed in Set-1. Stress appears to be one of the factor responsible for the decline in TEC through impairment of erythropoietic machinery of fish (Terassoli 1975, Das & Mukherjee 2000 a & b). During second half of the experiment,

Table 3: Percental increase of Total leucocyte count (TLC) of *Labeo boga* exposed to different sublethal concentrations of Lindane

	Different Sublethal Concentrations		
Exposure period	**Set I (10%)**	**Set II (20%)**	**Set III (30%)**
1-5 Days	13.51%	21.05%	23.80%
5-10 Days	16.19%	16.52%	15.38%
10-15 Days	8.60%	5.22%	8.33%
15-20 Days	2.64%	3.54 %	3.07%
20-25 Days	1.44%	1.71 %	2.08%
25-30 Days	0.72%	0.67%	1.16%

Table 4: Alterations in the Total Erythrocyte Count (TEC) (x 10^6 Cells/mm^3) of *Labeo boga* exposed to different sublethal concentrations of Lindane

	Different Sublethal Concentrations		
Exposure period	**Set I (10%)**	**Set II (20%)**	**Set III (30%)**
Control	2.5	2.5	2.5
1 Day	2.45±0.11	2.4±0.08	2.32±0.21
5 Days	2.30±0.32	2.22±0.09	2.01±0.80
10 Days	2.18±0.48	2.03±0.52	1.70±0.16
15 Days	2.05±0.42	1.85±0.57	1.30±0.70
20 Days	1.99±0.41	1.80±0.92	1.15±0.86
25 Days	1.94±0.41	1.75±0.96	1.10±0.72
30 Days	1.90±0.42	1.64±0.76	1.02±0.26

Table 5: Alterations in the Haemoglobin(Hb%) of *Labeo boga* exposed to different sublethal concentrations of Lindane

	Different Sublethal Concentrations		
Exposure period	**Set I (10%)**	**Set II (20%)**	**Set III (30%)**
Control	6.2	6.2	6.2
1 Day	6.2±0.13	6.2±0.09	6.2±0.75
5Days	6.2±0.12	6.0±0.12	6.0±0.81
10 Days	5.9±0.16	5.6±0.18	5.5±0.92
15 Days	5.6±0.17	5.3±0.08	5.0±0.48
20 Days	5.5±0.11	5.2±0.38	5.0±0.96
25 Days	5.5±0.34	5.2±0.72	4.8±0.17
30 Days	5.4±0.32	5.0±0.82	4.7±0.99

Table 6: Alterations in the Total Leucocyte Count (TLC) (x 10^6 Cells/mm^3) of *Labeo boga* exposed to different sublethal concentrations of Lindane

Exposure period	Set I (10%)	Set II (20%)	Set III (30%)
Control	9.25	9.25	9.25
1 Day	9.25±0.45	9.50±0.15	10.5±0.44
5Days	10.5±1.05	11.5±1.64	13.0±1.06
10 Days	12.2±.48	13.4±1.52	15.0±1.46
15 Days	13.25±0.76	14.1 ±0.61	16.25±0.56
20 Days	13.6±1.49	14.6±0.65	16.75±1.86
25 Days	13.8±1.07	14.85±1.56	17.1±1.44
30 Days	13.9±1.09	14.95±0.42	17.3±0.76

Table 7: Alterations in the Haemocrit / packed cell Volume (%) of *Labeo boga* exposed to different sublethal concentrations of Lindane

	Different Sublethal Concentrations		
Exposure period	Set I (10%)	Set II (20%)	Set III (30%)
Control	30.2	30.2	30.2
10 Days	27.6±0.75	24.3±1.03	20.5±0.72
30 days	23.5±0.32	19.8±0.81	15.4±1.69

Table 8: Alterations in the mean corpuscular Haemoglobin(MCH)(Pg) of *Labeo boga* exposed to different sublethal concentrations of Lindane

	Different Sublethal Concentrations		
Exposure period	Set I (10%)	Set II (20%)	Set III (30%)
Control	2.48	2.48	2.48
1 Day	2.53	2.58	2.67
5Days	2.69	2.70	2.98
10 Days	2.70	2.75	3.23
15 Days	2.73	2.86	3.84
20 Days	2.76	2.88	4.34
25 Days	2.83	2.97	4.36
30 Days	2.84	3.04	4.60

Table 9: Alterations in the mean corpuscular Haemoglobin Concentration (MCHC)(%) of Labeo boga exposed to different sublethal concentrations of Lindane

	Different Sublethal Concentrations		
Exposure period	Set I (10%)	Set II (20%)	Set III (30%)
Control	20.52	20.52	20.52
10 Days	21.37	23.04	26.82
30 Days	22.97	25.25	30.51

Table 10: Alterations in the mean corpuscular Volume (MCV)(µm3) of *Labeo boga* exposed to different sublethal concentrations of Lindane

Exposure period	Different Sublethal Concentrations Set I (10%)	Set II (20%)	Set III (30%)
Control	48.32	48.32	48.32
10 Days	58.07	58.96	70.93
30 Days	65.09	73.61	148.01

gradual decline in TEC is observed up to 15-25 days (Table 1) in Set-2 and Set-3 which according ton the present author may be due to the fact that the fishes have now revived and started synthesis and subsequent release of erythrocytes into the circulation. Fresh rise of decline observed from 20-30days in Set-3 and 25-30days in Set-1 and Set-2 may be an outcome of another phase of stress of insecticide. The smear preparation of blood (Fig. 3) further indicate that the erythrocytes not only decline

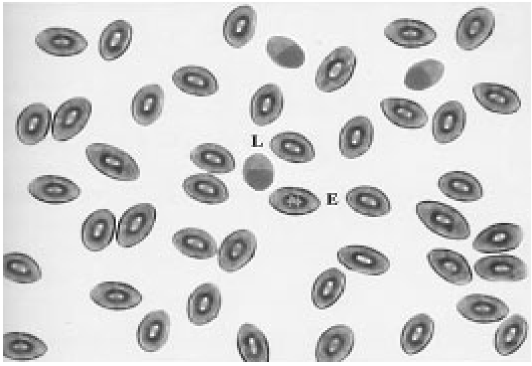

Fig. 1: Microphotograph of blood smear showing lymphocytes (L) and Erythrocytes (E)

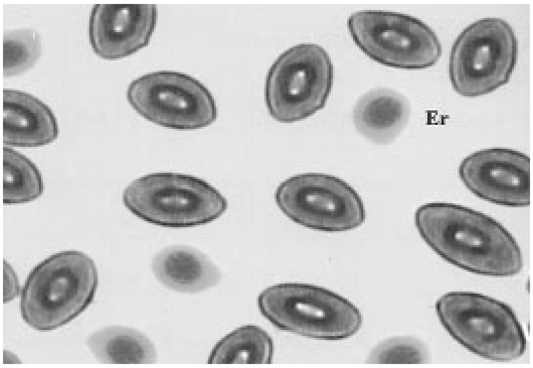

Fig. 2: Microphotograph of blood smear showing immature erythrocytes (Erythroblasts, Er)

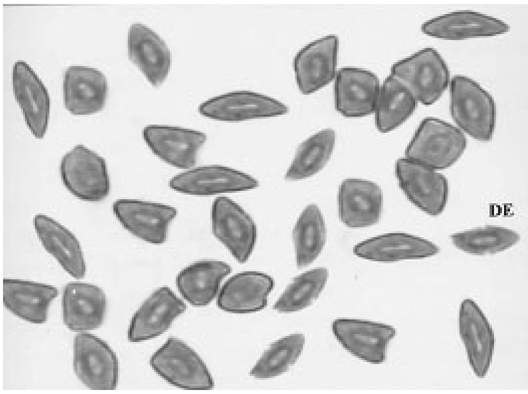

Fig. 3: Microphotograph of blood smear showing distorted erythrocytes (DE)

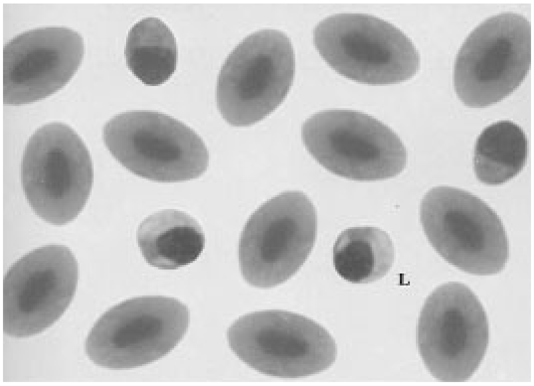

Fig. 4: Microphotograph of blood smear showing increased number of lymphocytes (L)

quantitavely bit qualitatively too i.e. Erythrocytes get deformed by abnormal shape. Numerical loss of erythrocytes may cause an imbalance in the respiratory physiology of the fish by reducing the surface area of erythrocytes and hence haemoglobin concentration (Das 1998, Verma 2007, Sachar 2008, Gupta 2008).

Similar to TEC, Haemoglobin also exhibited decline in their percentage throughout the experimental period of 30days except during 15-20days (Table 2 & 5) in Set3 and 20-25days in Set-1 and Set-2, where negligible decline have been observed in the haemoglobin percentage of the fish.

Similar to TEC and Hb, haematocrit (Hct) as expected follow the same trend of decline in all the experimental sets (Table). Decline in Hct (Table 7) content of the fish can be attributed to either erythrocyte lysis or release of greater number of erythroblasts (immature erythrocytes) into the general circulation (Srivastava and Mishra 1979).

Contrary to TEC, Hb and Hct, an increase have been observed in the calculated values of mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) (Table 8, 9 & 10) in all the experimental sets (table).

Increase in MCV may be taken as an index of RBC destruction whereas MCH and MCHC indicate cell swelling (Tyagi and Srivastava 2005).

Due to significant fall of Hb, RBC and Hct and increase in MCH, MCV and MCHC, two types of anemia have been reported for *Labeo boga* of present studies.

1. Hypochromic microcytic anemia:- which is associated with iron deficiency and decrease utilization for haemoglobin synthesis (Natarajan 1981)
2. Megaloblastic anemia:- which finds relation with prevalence or occurrence of high percentage of erythroblasts in the circulation (fig.2))

Leucocytes

Total Leucocyte Count (TLC)

From the pattern of increase in total leucocyte count (TLC) as recorded in all the sets of the experiments (table 3 & 6), it could be very safely inferred that toxic substances present in insecticide lindane might be responsible for the stimulation of lymphopoiesis in the lymphomyeloid tissue particularly spleen thereby releasing more and more lymphocytes (fig. 4) in the circulation. Such response of lymphocytes has already been also associated with pollutant induced tissue damage (Haniffa 1990, Meenakala 1978). Explaining drastic decline in the magnitude of increase of TLC during last end days of the experiment, the author intends to state that by this time, the fish appear to have becomes habitual to sustain itself in this sublethal concentration of the toxicant and hence the fish no longer appears to require any further increase of lymphocytes to cope up with the stress to which it has become used to. Hence fall in percental increase of TLC gets justified.

CONCLUSION

It can be concluded from the above results that exposure of *Labeo boga* to even sublethal concentration of lindane result in marked changes in the haematological parameters. Such changes although generally go unnoticed in the natural environment but their impact on human beings are often overwhelmed.

It thus becomes important that the effluents containing pesticides/insecticides be disposed as for as possible after their proper treatment for which certainly strict and regular monitoring of such toxicants in the water bodies is urgently required lest we loose our natural assets.

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