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Toxic Effect of Chlorine on Selected Blood Parameters of the Fish *Mystus Montanus*

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***Abstract:*-- Presently, there is a steady increase in the application of unlimited dose of chlorine to control vectors of Dengue, Malaria, Chickun-guinea etc., in village water sources. To analyse the toxic effects of chlorine on selected hematological parameters of the fish Mystus montanus a study was conducted for 45 days. Chlorine increased the concentration of WBC and decreased the concentration of RBC and Haemoglobin in a dose dependent manner. At high dose of 0.132mg Cl/L, the WBC was increased to 38.69 % and the RBC and Hb were decreased to 35.44 % &27.93% respectively from the control group of fishes. Haemolysis and vacuolated RBCs were observed in all the experimental group of fishes. Addition of thiosulphate, prolonged aeration and substitute for chlorine could be used to overcome the problems caused by chlorine.**

***Keywords:*-- Chlorine, Mystus montanus, Blood parameters, Toxic effects, Suggestive measures.**

1. INTRODUCTION

Elemental chlorine (Cl2) and other chlorine compounds have been used as disinfectants in water purification and wastewater treatment for many years because they are effective, relatively inexpensive, and remain active within systems for a considerable length of time (6).

In water at pH values above 5, elemental chlorine reacts rapidly. This reaction results in the formation of hypochlorous acid (HOCl) in equilibrium with hypochlorite ions (OCl-), known as 'free chlorine' (3). At pH 7 and a temperature of 25°C, 70 % of chlorine will be present as HOCl and at pH 8, 80% of chlorine will be present as OCl- (10). Aquatic organisms tend to be more sensitive to chlorine at higher temperatures and so added care may be warranted when chlorine is present in heated water discharges (10)
In general, recommended concentrations of residual chlorine for disinfection are between 0.5 and 1.0mg/l. For warm water fish there is a narrow range of acute lethal chlorine concentrations. The 96 hours LC50 for black bull head is 0.99mg /l and 15 hours LC50 for small mouth bass was reported to be 0.5mg /l (1). Chlorine in water destroys protective acidophilus, which nourishes and cooperates with the 3 to 3.5 pounds of immunity-strengthening "friendly" organisms lining the colon, where about 60 percent of our immune cells operate (4). Chlorine combines with organic impurities in the water to make trihalomethanes (THMs), or chloramines. Among the THMs that result from chlorine combining with organic compounds in water are carcinogenic chloroform and carbon tetrachloride (7).

Hematological tests are important diagnostic tools and recent findings have suggested that they may be equally valuable as indicators of disease or stress due to pollutants and environmental fluctuations in fishes (2). Further, hematological observations have greater contribution to the pathological changes obtained during toxicological studies. Hence, the present investigation was carried out to corroborate the effect of chlorine on selected haematological parameters and on red blood cell morphology of fresh water fish Mystus montanus.

II. MATERIALS AND METHODS

A. Calculation of LC^{50} Value:

The source of chlorine was commercially available sodium hypochlorite. To find out the LC^{50} for chlorine, a series of 10 concentrations ranging from 0.10mg/L to 1mg/L were prepared separately from the stock toxicant. Well acclimatized 10 fish of uniform size were selected and introduced into each toxicant concentration taken in 20 liter capacity plastic buckets for a period of 96 hours. Every day the fresh concentrations were prepared from the stock, to make the concentrations constant throughout the experimental study (11). During the experimental period the fish was not fed. The mortality of test individuals in each toxicant concentration was recorded. The experiment was repeated twice, simultaneously to confirm the result. During the experiment, a control group of fish was also reared. The LC50 value of Chlorine to the fish Mystus montanus.for 96 hours was calculated as 0.66 mg/L

B. Rearing of fish

Mystus montanus fish with an individual mean weight of about 6 ± 0.5g were acclimatized in the tub for one week.

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The fishes were fed with Commercial pellet feed once in the evening at 5% body weight for 45 days. The water in the fish culture tub was changed daily after feeding. The fish were distributed randomly among four glass tanks (for clear vision of pathologic conditions) of 25 liter capacity at a stocking density of 10 per tank with treatment in triplicates. One group served as control. The other three experimental groups of fish were exposed to 0.033mg Cl/L (5% of LC50), 0.066mgCl/L (10% of LC50) and 0.132mgCl/L (20% of LC50).

Table-1: Experimental Design

Fish group	No of fish	Treatment	Duration	Feeding %
Control	10	Tap water	45days	5% body mass
Group I	10	water with 0.033Cl/L	45 days	5%body mass
Group II	10	water with 0.066Cl/L	45 days	5% body mass
Group III	10	water with 0.132Cl/L	45 days	5% body mass

C. Collection of Blood:

The fish was suspended on a hook by its mouth. The tail is cut off with a pair of scissors. The dripping blood is collected and used for hematological studies such as total RBC, WBC and Hemoglobin.

D. Estimation of RBC, WBC and Hemoglobin:

The RBC and WBC were counted by using improved Neubar counting chamber. Haemoglobin was estimated by using haemoglobinometer. A thin blood smear was prepared on a clean slide and stained with Wright's stain. The morphology of blood cells was photographed in the high power (45X × 15X) of compound microscope.

III. RESULTS

Haematological parameters of Mystus montanus such as number of RBC, WBC and Haemoglobin exposed to three sub lethal concentrations of chorine for 45 days showed the results as indicated in Table: 2.

Table.2: Haematological parameters of Mystus montanus exposed to chlorine

Sample	RBC 10^6/mm^3	WBC 10^3/mm^3	HB mg/dl
Control	3.37 ±0.13	3.47 ±0.12	5.37 ±0.12
Group I	2.93 ±0.12	3.8 ±0.081	4.93±0.1249
Group II	2.53 ±0.11	4.06 ±0.122	4.5 ±0.163
Group III	2.37 ±0.12	4.7 ±0.161	3.87 ±0.18

A. RBC:-

The RBC count of the control fish was 3.37±0.13 x10^6 /mm^3. The RBC count was decreased to 2.93±0.12x10^6 /mm^3, 2.53±0.11 x10^6 / mm^3 and 0.10±0.120x10^6 /mm^3 respectively in 0.033 mg Cl/L and 0.066 mg Cl/L and 0.132 mg Cl/L exposed fish groups.

B. WBC:-

The WBC count of control group of fish was 3.47±0.12x10^3 /mm^3. Chlorine exposure increased the RBC count to 3.8±0.081 x10^3 /mm^3, 4.06±0.122 x10^3 /mm^3 and 4.7 ±0.161 x10^3 /mm^3 in the fish groups treated with 0.033 mg Cl_2/L, 0.066mg Cl_2/L and 0.132mg Cl_2/L respectively.

C. Haemoglobin:-

The Haemoglobin concentration of control fish was 5.37 ±0.12 mg Cl/L. The Haemoglobin concentration in the blood of experimental fish was decreased than the control group of fish.

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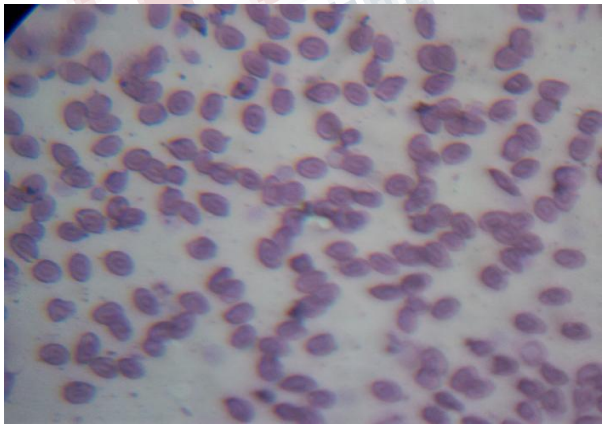
Table.3: Comparison of Hematological parameters of Mystus montanus

Comparison	Decreasing % of RBC	Increasing % of WBC	Decreasing % of HB
C Vs Group I	13.05 %	9.5%	8.19 %
C Vs Group II	25.00 %	17.00 %	16.20 %
C Vs Group III	38.69 %	35.44 %	27.93 %

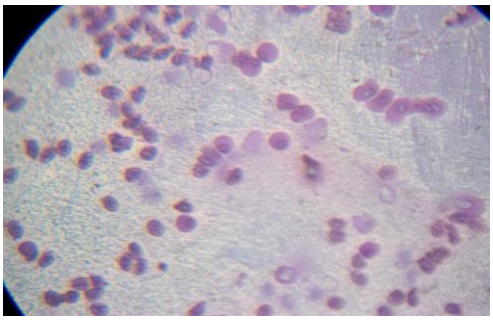
D. Microscopic observations of RBC:-

The blood smear of Fish exposed to three different sub lethal doses of fish are described below. The control group of fish showed RBC with normal structure and morphology. No haemolysis and vacuolation was observed (plate.1).

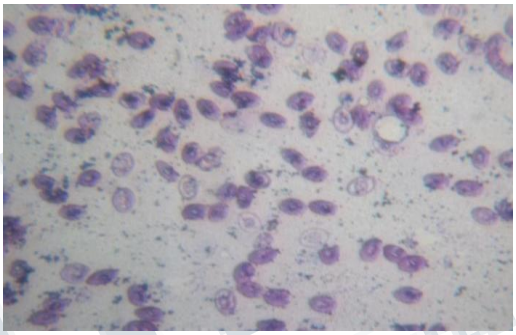
The fish exposed to 0.033mg Cl/L showed some hemolytic RBC. Other RBC showed normal structure (Plate 2). Fishes exposed to 0.066mg Cl/L showed wrinkling of membrane, haemolysis and fragile Red blood corpuscles (Plate -3). The RBC of 0.66mg Cl/L exposed fish was vacuolated and 75% of the RBC was fragile. The size of the cell was reduced and the RBC was irregular shaped and vacuolated (plate -4).



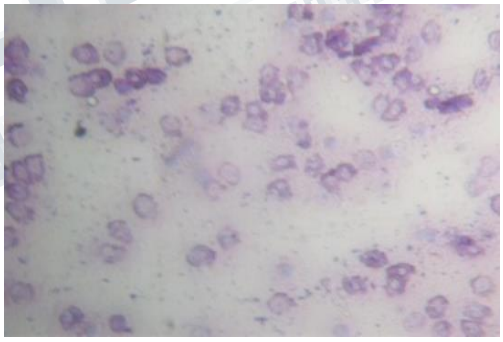
Plate, 1 shows normal RBCs



Plate,2 shows some haemolytic RBCs



Plate,3 shows haemolytic & vacuolated RBCs



Plate,4 shows haemolytic & shrunkened RBCs

IV. DISCUSSION

The present study was designed to analyse the effect of chlorine on the haematology of the fish Mystus montanus. The total RBC and haemoglobin were reduced in Mystus montanus on exposure to three different sub lethal concentrations of chlorine. The RBC count and Hb were decreased gradually when the concentration of chlorine was increased in the fish. The RBC count and haemoglobin was decreased in the three experimental groups of fishes. The results recorded in this investigation substantiate the previous findings in fishes by (8) after exposure to cypermethrin.

The white blood corpuscles of Mystus montanus showed increase and quite different pattern of changes with the effect

of chlorine when compared with the erythrocyte levels of the control group. Blood of all experimental groups contained higher concentrations of leucocytes than those of controls. Pooja Gupo and Kumar saxena, (9) observed increased WBC counts in Channa punctatus after cyhalothrin and permethrin. They have suggested that significant increase of WBC in the fish could be due lympocytopoeis and enhanced release of lymphocytes from lymphoid tissues such as kidney spleen and thymus to cope up the toxic condition.

In the present study the blood smear of fish exposed to three different sub lethal doses of chlorine after 45 days showed number of fragility, vacuolation, wrinkling of membrane, and haemolysis of erythrocyte cells. A distinct change in the size and irregular shape of red blood cells was also observed. Devip et al., (5) also observed similar findings in fish, Channa punctatus treated with pesticide endosulfan. Thus, the present study concludes that chlorine is highly toxic like pesticides such as cypermethrin, cyhalothrin and permethrin to the fish Mystus montanus at a concentration below 0.0332mg/l.

V. SUGGESTION

Less than one-half (0.5) mg/l of free chlorine is needed to kill bacteria without causing water to smell or taste. But during diseases outbreak the water sources in the rural villages disinfected with a very high dose of chlorine that leads to not only unpleasant smell and also throat and stomach ailments to the consumer. To overcome these problems the following remedial measures can be helpful.

- Prescribed dose of chlorine should be used for disinfection. In areas receiving wastes treated continuously with chlorine, not to exceed 0.01 mg/l for the protection of more resistant organisms only, or not to exceed 0.002 mg/l for the protection of most aquatic organisms (12).
- Chlorinated solutions, in which the toxicity is due to free chlorine, may be rendered nontoxic by treatment with sufficient thiosulphate to discharge residual chlorine.
- Prolonged aeration greatly reduce the toxicity by displacing residual chlorine in the water.
- If free chlorine persists, more restrictive criteria are warranted. Alternate procedures or substitutes for chlorination should be investigated.

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