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IMPACT OF PETROCHEMICAL EFFLUENT ON HEMATOLOGICAL PARAMETERS IN THE FRESHWATER FISH, *LABEO ROHITA*

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ABSTRACT: The present investigation was carried out the effect of petrochemical effluent on hematological parameters in freshwater fish, *Labeo rohita* using the standard method. The petrochemical effluent has the potential to cause developmental effects in fish. Blood offers important profile to study the toxicological impact on animal tissues. Hematology is used as an index of fish health status under different stress conditions on exposure to pollutants. The fish *Labeo rohita* was exposed to sub-lethal concentrations (30 ml/L) of petrochemical effluent for different h (24, 48, 72 and 96) and parameters like RBC, WBC, HB, MCV, MCH MCHC, PCV has been analyzed. All hematological parameters except WBC were found to be decreased from control, and the WBC was increased in all exposure periods indicating that test fish, *Labeo rohita* suffered hemolytic anemia and leucocytosis. Increase in time produced a declining value of RBC, Hb, MCV, and MCH also exhibit the sub-lethal concentrations of the petrochemical effluent.

INTRODUCTION: Water pollution is the contamination of water bodies. This form of environmental degradation occurs when pollutants are directly or indirectly discharged into water bodies without adequate treatment to remove harmful compounds. The agrochemicals and industrial discharges may be carried away effectively by rains, winds, rivers, and floods into the large water bodies and change their physic-chemical properties with high toxicity. The water contamination cause damages to aquatic life, especially to fishes, which are very sensitive to a wide range of toxicant in the water.

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The rapid industrial growth throughout the world particularly due to the alarming rise in human population has been responsible for a tremendous amount of environmental pollution. In developing countries, industrial effluents are indiscriminately discharged into aquatic ecosystems and even into adjoining fields without any pre-treatment, thus creating serious problems to the nontarget organisms.

Discharging of effluents into freshwater systems depletes the dissolved oxygen content causing the heavy mortality in fish by interfering with respiratory metabolism. The hematological studies in fishes have assumed greater importance because these parameters were used as an efficient and sensitive index to monitor the physiological and pathological changes induced by nature or anthropogenic factors such as bacterial or fungal infection or pollution of water resources. Study of the blood in fishes is therefore important from the

diagnostic point of view. The hematological effects have been reported in fishes due to various toxicants by several investigators. Blood parameters therefore considered a useful tool in diagnosing the functional status of the body in response to various stressors. The toxicants are stressors which are accumulated in the fish through the food chain or absorption through the general body surface and severely affect the life-supporting system at the molecular and biochemical level. The pollutants generally produce relatively quick changes in hematological characteristics of fish.

Blood cell count is a stable index, and normally fish tries to maintain it between certain limits. But the presence of toxicants in water may lead to a change in water quality, which may ultimately change one or more hematological parameters. Fish blood is very important to accurately evaluate the health of species. Blood parameters are considered pathophysiological indicators of the whole body and therefore are important in diagnosing the structural and functional status of fish exposed to toxicants [1].

The study of fish blood parameters is important for determining factors related to their physiological capacity. Blood parameters are considered path physiological indicators of all body. Several hematological parameters such as RBC, WBC, and hemoglobin content and so on, are used to assess the functional status of the oxygen-carrying capacity of the blood. The release of chemicals into the aquatic environment results in some changes, which may threaten functional attributes, the integrity, and existence of aquatic organisms, especially fish. Recently, hematological parameters have become promising biomarkers in measuring the effects of chemical pollutant in fish. Blood samples can regularly be obtained from test organisms, thus allowing the use of non-destructive approach in effecting assessment [2].

Hematology is used as an index of fish health status in number of fish species to detect physiological changes following different stress conditions like exposure to pollutants, diseases, metals, hypoxia, etc. in order to use these blood parameters with a diagnostic purpose, it is necessary to analyze healthy fish in the best ambient conditions to establish the reference indices for the species object of study, as well as to analyses fish under different stressed conditions and establish specific variation in each parameter, compare it with the normal values and make a diagnosis. Blood parameters play a significant role in blood characteristics. The fish blood is an analytical tool in establishing the health position of the fish [3]. It helps in evaluating the response of different types of blood cells and its components in the conditions of physiological stress owing to toxicity, as it speedily reflects the deprived situations of fish than other generally measured parameters.

MATERIALS AND METHODS: Bulk of sample fishes, *Labeo rohita ranging* in weight from 7-10 gm and measuring 5-8 cm in length were procured from Aliyar Dam. Fishes were acclimatized to laboratory conditions, for 21 days in a large syntax tank. During the period of acclimatized, they were fed every day with rice bran and oil cake 1:1 ratio. Feeding was stopped one day before start the experiment, to keep the animal more or less in the same state of metabolic requirement. Fishes about the same size irrespective of sexes were selected for the experiment. Batches of healthy fishes were exposed to different concentrations of petrochemical effluent to calculate LC_{50} value. LC_{50} value was found as 30 ml for 96 h using probit analysis method [4].

Four groups of fishes were exposed to 30 ml (sub-lethal concentration of 96 h LC_{50} value) concentration of the petrochemical effluent for 24, 48, 72, 96 h respectively. Another group was maintained as control at the end of each exposure period the blood was collected from gills. Using syringe and anticoagulant were added, and hematological parameters were analyzed. The RBC and WBC were counted by hemocytometer, Hb was estimated by acid haematin method [5]. PCV was calculated employing the standard method and formulate [6]. The mean corpuscular volume was calculated by using values of PCV% and the red blood cell counts expressed in μm^{-3}. [7] The mean corpuscular hemoglobin content was calculated by using the value of hemoglobin content, and the red blood cell counts and expressed in pg.

RESULTS AND DISCUSSION: In the present study, the effect of petrochemical effluent on hematological parameters such as RBC, WBC, Hb,

MCV and MCH in the freshwater fish, *Labeo rohita* have been studied and tabulated **Table 1**. The amount of RBC in the blood of the fishes exposed to 30 ml petrochemical effluent for 24, 48, 72 and 96 h was found to contain 1.72, 1.65, 1.50, 1.42 × 10^6/mm^3 and mean control was found to be 1.85×10^6/mm^3. The amount of WBC was found to be increased from the control. The values were 2.73, 2.87, 3.24, 3.62 and 2.64 × 10^6/mm^3 in control 24, 48, 72 and 96 h respectively. The level of hemoglobin in the fish, *Labeo rohita* on exposed to 24, 48, 72 and 96 h was found to contain 3.00, 2.85, 2.06, 1.8 gm % and mean control was found to be 3.20 gm %. The amount of PCV in the blood of the fishes exposed to 30 ml petrochemical effluent for 24, 48, 72 and 96 h was found to contain 11.00, 10.3, 9.1, 8.3 and mean control was found to be 11.32%. The value of MCV in fishes exposed to 30 ml petrochemical effluent for 24, 48, 72 and 96 h was found to contain 42.3, 40.1, 38.4, 30.40 μm^3 and mean control was found to be 45.10 μm^3. The amount of MCH in the blood of the fishes exposed petrochemical effluent was recorded as 18.62, 17.20, 15.00, 14.80, and the control was found to be 19.40 Pg.

TABLE 1: EFFECT OF PETROCHEMICAL EFFLUENT ON HAEMATOLOGICAL PARAMETERS IN BLOOD OF THE FRESHWATER FISH, *LABEO ROHITA*

Blood parameters	Exposure periods				
	Control	24h	48h	72h	96h
RBC	1.85 ± 0.08	1.72 ± 0.03	1.65 ± 0.46	1.50 ± 0.42	1.42 ± 0.39
't' value		4.89**	6.63**	7.35**	8.48**
% Change		-7.027	-10.810	-18.918	-23.243
WBC	2.64 ± 0.10	2.73 ± 0.15	2.87 ± 0.65	3.24 ± 0.53	3.62 ± 0.41
't' value		6.28**	6.61**	7.53**	8.39**
% Change		3.409	8.712	30.681	37.121
Hb (g %)	3.20 ± 0.07	3.00 ± 0 .58	2.84 ± 0.63	2.06 ± 0.50	1.8 ± 0.23
't' value		5.13**	7.28**	8.15**	9.08**
% Change		-6.25	-11.25	-35.625	-43.75
PCV (%)	11.32 ± 0.08	11.00 ± 0.08	10.3 ± 0.11	9.1 ± 0.05	8.3 ± 0.09
't' value		20.27**	21.28**	22.22**	23.18**
% Change		-2.826	-9.010	-19.611	-26.678
MCV(μm^3)	45.10 ± 0.64	42.3 ± 0.62	40.1 ± 0.92	38.4 ± 0.85	30.40 ± 0.59
't' value		55.27**	58.28**	59.22**	60.18**
% Change		-6.208	-11.086	-14.855	-32.594
MCH (pg)	19.40 ± 0.14	18.62 ± 0.52	17.20 ± 0.42	15.00 ± 0.35	14.80 ± 0.29
't' value		25.27**	26.28**	27.22**	29.18**
% Change		-4.020	-11.340	-22.680	-23.711

Results are mean (±SD) of 5 observations, % = Parenthesis denotes percentage increase/decrease over control.

Reduction in the RBC due to toxicant exposure Level of petrochemical effluent exposed for some time can reduce the erythrocyte production. It has been reported that the decrease in RBC leads to the hemolytic crisis and result in severe anemia in fish exposed to petrochemical effluent. The decrease in RBC in the present investigation might be resulted from the inhibition of RBC production or due to the accumulation of effluent in the gill region causing damage in the structure of the gill resulting in hemolysis [8]. The increase rate of the breakdown of RBC or reduced rate of formation of RBCs might also be responsible for the reduction in RBC count. Similar observations were reported for *Clarias gariepinus* treated with endosulfan pesticides [9]. The reduction may be due to an increased rate of breakdown of red blood cells and reduction in the rate of formation of red blood cells [10]. The reduced erythrocyte parameters are indications of macrocytic anemia emanating from increase destruction and subsequently enhanced erythropoiesis in the liver.

The increase in WBC in stressed animals is a protective response to stress [11]. The WBC count increases in the fish with time duration. This may be the attribution to the induction of some defense mechanism in the body of fish to tide over the pollution stress or might be due to an immunological reaction to produce more antibodies to cope with the stress induced by the toxicant. Total WBC count and leucocrit increased in *Tinca tinca* exposed to lethal and sub-lethal treatments with mercury [12]. Increase in the leucocytes number

in fish, *Hoplias malabaricus* exposed to subchronic and dietary doses of methyl mercury [13]. Increase in WBC count occurred as a pathological response since these WBCs play a great role during infestation by stimulating the hemopoietic tissues and the immune system by producing antibodies and chemical substances working as a defense against infection [14].

The reduction in hemoglobin content in fish exposed to toxicant could also be due to the inhibitory effect of the toxic substance on the enzyme system responsible for the synthesis of hemoglobin. Depletion in hemoglobin was noticed in fish exposed to petrochemical effluent, and it may be due to the lysis of erythrocytes [15]. The decrease in Hb corresponds with the decrease in dissolved oxygen; an indication that the decrease in hemoglobin resulted in haemodilution. The Hb values fall lower than the range reported for catfish [16]. The reduction may be due to an increased rate of breakdown of red blood cells and reduction in the rate of formation of red blood cells [10].

The low Hb recorded mighty be as result influx of water from the farms, industrial, sewage that contained heavy metals such as cadmium, nickel, and lead, which alter the properties of hemoglobin by decreasing their affinity towards oxygen binding capacity rendering the erythrocytes more fragile and permeable [17]. The above findings supported the present observation that the reduction of hemoglobin due to toxicants leads to a significant decrease in hemoglobin, finally reduction in oxygen binding capacity of fishes. Reduction in Hb content indicates that decline in Hb synthesis as well as a reduction in oxygen carrying capacity, which may perhaps be as a result of interference of petrochemical effluent with haem or globin synthesis pathway.

The study revealed considerable variation in the PCV, MCV, and MCH in the fish exposed to petrochemical effluent at different time duration in the laboratory condition. It was observed that the MCV and MCH changed significantly at higher concentration. Similar observations found in *C. gariepinus* exposed to formalin [18]. Various researches reported that the cells released from the spleen, the erythropoietic organ might have lowered the MCV value [19]. The high percentage of immature red blood cells in circulation might be the reason for the decreased MCV and MCH [8]. The mean corpuscular hemoglobin concentration which is the ratio of the mean hemoglobin concentration is not influenced by blood volume either by the number of cells in the blood but can be interpreted incorrectly only when new cells, with a different hemoglobin concentration, are released [20].

The PCV represents the percentage of cell present in the blood. Decreased in PCV% was observed in treated fishes during the present investigation. This could be due to the toxicants presents in polluted water influence the malfunctioning of the hemopoietic system. Therefore the hemopoietic tissues fail to release the blood cells, which subsequently release into the blood system. The decrease in PCV may show the extent of shrinking cell size and decrease in the number of cells due to Danitol intoxication in the fish, *Ctenopharyngodon idella* [21]. Diazinon exposure in carp led to a decrease in PCV, and their results are similar to the present study [22]. The low value of PCV in fish exposed to stress was attributed to a reduction in red blood cell volume caused by osmotic changes [4]. The decrease in MCV and PCV values in the fish, *Channa punctatus* due to the effect of Rayon industry effluents. All these observations confirm the findings of the present study [23].

CONCLUSION: Blood offers important profile to study the toxicological impact on animal tissues. Different blood parameters are often subjected to change depending upon stress condition and various other environmental factors. Decrease or increase in certain blood parameters can be associated with the nature of species and the toxicants in different studies. The decrease in hematological variables (PVC, Hb, RBC) of the exposed fish may be due to hematological variables (PCV, Hb, RBC) of the exposed fish may be due to hemolysis and shrinkage of RBC by petrochemical effluent leading to significant disease in hematocrit value which results in fish anemia.

The hematological parameters except WBC were found to be decreased from control, and the WBC has increased in all exposure period. From the above investigation, it can be inferred that the aquatic animals are affected by the petrochemical effluent discharge *via* effluents into aquatic

environments caused severe anemia and alterations in hematological indices in the freshwater fish, *Labeo rohita.*

ETHICAL APPROVAL: We do not require ethical approval for carrying out experiments with fishes in India.

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CONFLICT OF INTEREST: The authors have declared no conflict of interest

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