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# Variation In Haematological Parameters After Toxicity Of Pesticide Polo In Blood Of Freshwater Fish, *Labeo rohita*

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**Abstract**:
In thepresent investigation, effects of Broad Spectrum Insecticide Polowere analyzed in blood of freshwater fish, *Labeorohita*after 7 to 21 days chronic exposure at laboratory condition. $LC_{50}$ of pesticiede Polo for 96hrs was 1.9774ppm. Fishes were exposed to sublethal concentration as $LC_{50/2}$ (0.9887ppm). Blood parameters like, Haemoglobin (%), Haematocrit (%), total RBC's ($mm^3$), total WBC's ($mm^3$) count and blood indices like Mean Corpuscular Volume (MCVμm), Mean Corpuscular Haemoglobin (MCHpg) and Mean Corpuscular Haemoglobin Concentration (MCHC%) were assessed. Mean Corpuscular Haemoglobin Concentrations were increased as compare to control whereas all other blood parameters were decreased as compare to control in freshwater fish, *Labeorohita* during chronic exposure to an insecticide Polo.

**Keywords**:-Toxicology, *Labeorohita*, polo, haematological parameters

**Introduction**: Blood parameters are considered as good physiological indicators of the whole body conditions and therefore can be used in diagnosing the structural and functional status of fish exposed to toxicants (Adhikarietal., 2004; Serianietal., 2009). Blood indices are greatly used to evaluate the toxic stress of the fishes (Kavithaetal, 2010) and diagnosis of fish physiology are used to ascertain sublethal and chronic exposure of contaminants (Kim etal, 2008). Pollutions are major burning question throughout all over worlds today. Among all types of pollution , aquatic pollution is of greater concern as each and every kind of the life depends on water (Shuklaetal, 2009). Organophosphate pesticides are largely used in agroecosystems for killing insect pests and to increase greater enhancement of crops because among various groups of pesticides, organophosphates are most frequently used, due to their high insecticidal property, low mammalian toxicity, less persistence and rapid biodegradibility in the environment (Srivastavetal, 2010). Rohu is candidate species in carp polyculture systems. The Indian major carp ***Labeorohita*** is a popular food fish in Khandesh region due to it's taste and flesh. So rohu had gain great economic importance as well as most demanding commercial fish. Hence, present investigation was undertaken to study the toxic effects of an insecticide **Polo** on haematologialparametres in blood of freshwater fish, ***Labeorohita***.

**Materials and Methods**:

The fish ***Labeorohita***were collected from Ganeshpur, Gadad and Girna river dams near Chalisgaon city, Dist. Jalgaon in Maharashtra, India. They were acclimated to laboratory conditions for 10-15 days in tank of 1000 liter, previously washed with Potassium permangnate and water. Physicochemical parameters of water were analyzed by following standard method of APHA (1985). The ambient temperature was 26.3 ± 35$^0$C and pH 7.0 – 7.2 maintained prior to subjecting them to experimentation. The acclimatized active and healthy fish were chosen for experiment. Two groups of these fishes were formed. Static bioassay studies were carried out by Finney's method (1971). One group was considered as experimental group exposed to reagent grade of Polo. Another group was without pollutants and was considered as control. $LC_{50/2}$ values of 96hrs were taken for chronic exposure (7 to 21 days). In this way, insecticide treated and control groups were made. Fish were fed regularly and water was changed every day in the control as well as treated group. The concentration of Polo remained the same within the experimental period. Proper aeration was maintained in test and control aquaria by air pump throughout experimentation.

Haematological parameters were assessed in five individual animals as per standard procedure. The blood from the control and treated fishes was obtained by using Kori-Siakpere and Egor, (1997) method and collected in Eppendorf tubes containing EDTA anticoagulant. These blood samples were used to estimate the haematological parameters like, Haemoglobin (Hb), total RBC and WBC count, Haematocrit (HCT%), Mean carpuscular volume (MCVmm), Mean carpuscularhaemoglobin (MCH pg) and Mean carpuscularhaemoglobin concentration (MCHC %) were studied.

**Blood Analysis**

**Estimation of Haemoglobin -** The haemoglobin (Hb) content of blood samples was determined by

**Cyanmethaemoglobin method** (**Wharton and McCarty 1972** ;**Blaxhall and Daisley 1973**). The microhaematocrit method of **Blaxhall and Daisley** (**1973**) is used for the determination of blood haematocrit.

**Total RBC's Count -** Total red blood cells (RBCs) count was done in an Improved Neubauerhaemocytometer following the method of **Baker and Silverton**, (**1982**).

**Total WBC's Count -** The total white blood cells (WBCs) count was determined in the same Improved Neubauerhaemocytometer (used for red blood cells) following the same method of **Baker and Silverton, (1982)**.

**Haematological Indices –**

Thehaematological indices like, Mean cell volume (MCV), Mean cell haemoglobin (MCH) and Mean cell haemoglobin concentration (MCHC) were calculated by using the formula of Baker and Silverton, (1982) as below-

**Mean carpuscularvolume(MCV μm)** = $\frac{\text{Haematocrit (\%)}}{\text{Erythrocyte count (mm}^3\text{)}}$ x 10

**Mean carpuscularhaemoglobin (MCH pg)** = $\frac{\text{Haematocrit(g\%)}}{\text{Erythrocyte count (mm}^3\text{)}}$ x 10

**Mean carpuscularhaemoglobin concentration (MCHC)%** = $\frac{\text{Haematocrit(g\%)}}{\text{Haematocrit (\%)}}$ x 100

**Results and Discussion:**

The haematological parameters alters after chronic (7 to 21 days) exposure to an insecticide Polo in blood of freshwater fish, ***Labeorohita*** as compared, to the control. The haemoglobin values decreased from **8.2** to **4.4** g%. HCT % significantly decreased from **27.06** to **11.88** %. The individuals treated with chronic exposure to Polo showed decrease in mean values of RBC's from **3.28** to **1.80** $mm^3$, the values mentioned above showed a significant decrease when compared to the control.

There was decrease in the total count of white blood cells of ***L. rohita*** after chronic exposure to Polo as compare to the control value. Total WBC count was decreased from **145.1** to **140.8** $mm^3$. The values mentioned above showed a significant decrease when compared to the control. The blood indices like, Mean corpuscular volume (MCVμm) decreased in freshwater fish, ***L. rohita*** from **82.5** to **66.0** μm after chronic exposure to Polo. The Mean corpuscular haemoglobin (MCH pg) also decreased in ***L. rohita*** from **25.0** to **24.44** pg after chronic exposure to Polo. The Mean corpuscular haemoglobin concentration (MCHC%) significantly increased in freshwater fish, ***L. rohita*** from **30.30** to **37.03**% after chronic exposure to Polo. Results of the study are summarized in table given below.

In the present investigation,depletion inhaemoglobin and haematocrit values in freshwater fish, ***Labeorohita***, after chronic exposure to Polo could be due to erythrocyte lysing. Significant reduction in these parameters is an indicating character of severe anaemia. Similar reduction has been reported by Samprathetal., (1993); Annuneetal., (1994b); Musa and Omoregie, (1999). Decreased haematocrit values may be an indication of haemodilution. Neumosok and Hughes (1998), observed haemoconcentration after copper exposure and haemodilution after zinc exposure in fish***, Colisafasciatus***; and Kori-Siakperee etal., (2008) observed haemodilution in ***Heteroclarias*** after zinc exposure. Decrease in haematocrit was reported by Tort and Torres, (1988), following 24 hrs exposure to cadmium in dogfish, ***Scyhorhinuscanicula*** and attributed this decreased haematocrit to haemodilution.

Depletion in haemoglobin and haematocrit values in present work coupled with decreased and deformed erythrocytes are obvious signs of anaemia. This is in agreement with Venkataraman and Sandhya Rani, (2013). Haemoglobin and haematocrit depletion was also noted by Hilmyetal., (1987) in catfish ***Clariaslazera*** and ***Tilapia zilli*** after zinc exposure; Veliseketal., (2009) in ***Oncorhynchusmykiss*** after exposure to bifenthrin.

Haematocrit appears to be positively correlated with RBC count. Therefore, it decreases. Destruction of erythrocytes (RBC's) triggered by the influx of pesticide Polo into the erythrocytes and might be cause anaemia and also be of haemolytic type of RBC. Similar results were reported by Tilaketal., (2007); Saravananetal., (2010); Saeedetal., (2012). In the present study, the total count of WBC's were decreased during chronic exposure of Polo in freshwater fish, ***Labeorohita***, could be due to defensive response against toxic stress.The decreased number of white blood cells may also be related to an increased level of corticosteroid hormones, whose secretion is a non-specific response to any environmental stressors (Iwamaetal., 1976; Ellis, 1981). Depletion in RBC, WBC count and haemoglobin level were noted previously by Christobheretal., (2016).

Decreased MCV values were noted in the present study. This may be due to erythropoetic organ, because spleen is an erythropoetic organ and it releases cells which lowers the MCV values. Similar observations were made by Koyama and

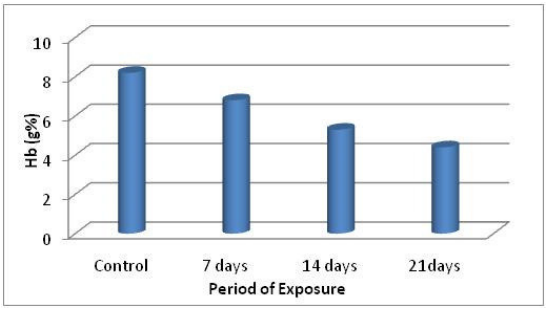
Ozaki, (1984). The decreased values of MCH in the present investigation may be also due to the reduction in cellular blood iron which reduces the oxygen carrying capacity of blood and eventually stimulates the erythropoesis. Similar observations were noted previously by Hodsonetal., (1978) in rainbow trout, ***Salmogardneri***; Kori-Siakpereetal., (2008) in ***Hetroclarias***species. Such a decrease in MCH value was also reported by Kudirat, (2007); Shah, (2006) under influence of heavy metals and pesticide stress in different fish species.

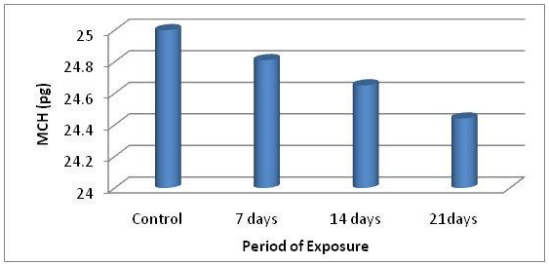
Mean corpuscular haemoglobin concentration (MCHC) was increased in the present investigation in fish, ***L. rohita*** after chronic treatment of Polo as compared to control. This may be due to alterations attributed to direct or feedback responses of structural damage to RBC membranes resulting in haemolysis and impairment in haemoglobin synthesis, stress related release of RBC's from the spleen and hypoxia. This is in agreement with the work of Shah, (2006); Venkatraman and SandhyaRaani, (2013). The RBC, Hb,Haematocrit, MCV, MCH and WBC in chronic exposure were decreased in the present study. This is in agreement with the work of Parikh,(2014); Mohammad Najedetal., (2012).

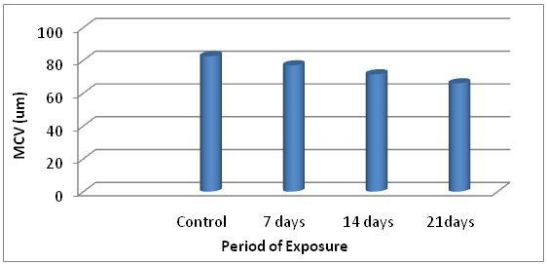
**Table-** Haematological parameters of blood of *L.rohita*after chronic exposure to Polo.

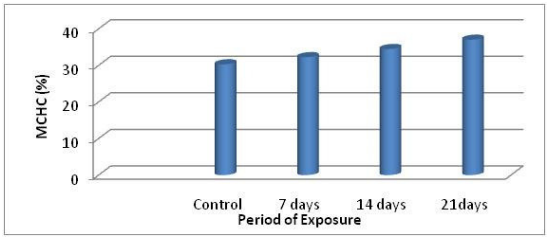
| Treatment | Hb(g%) | Haematocrit (%) | RBC ($mm^3$) | MCV (µm) | MCH(pg) | MCHC (%) | WBC ($mm^3$) |
|---|---|---|---|---|---|---|---|
| **Control** | 8.2 ±0.919** | 27.06 ± 0.256* | 3.28 ± 0.137* | 82.5 ±0.252** | 25.0 ±0.0303** | 30.30 ± 0.067* | 145.1 ± 0.313* |
| **Polo** | | | | | | | |
| **7 days** | 6.8 ±0.642** | 21.08 ± 0.0313* | 2.74 ± 0.083* | 76.93 ±0.0334*** | 24.81 ± 0.0547* | 32.25 ±0.0141** | 144.2 ±0.156** |
| **14 days** | 5.3 ± 0.0723* | 15.37 ± 0.026** | 2.15 ±0.055* | 71.48 ± 0.034** | 24.65 ±0.0331** | 34.48 ± 0.022** | 142.9 ± 0.182* |
| **21days** | 4.4 ± 0.618* | 11.88 ±0.0219** | 1.80 ±0.212* | 66.0 ±0.0178** | 24.44 ± 0.0173* | 37.03 ±0.0268** | 140.8 ±0.105** |

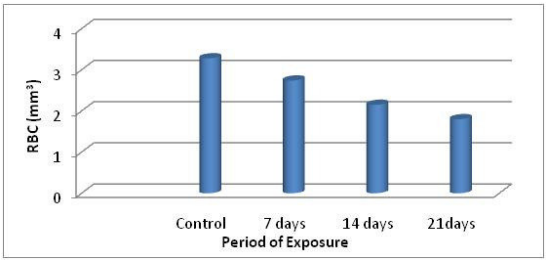
Each value is mean ± SD of five observations; * indicates significant at $p < 0.05$. ** indicates significant at $p < 0.001$. *** indicates significant at $p < 0.0001$.

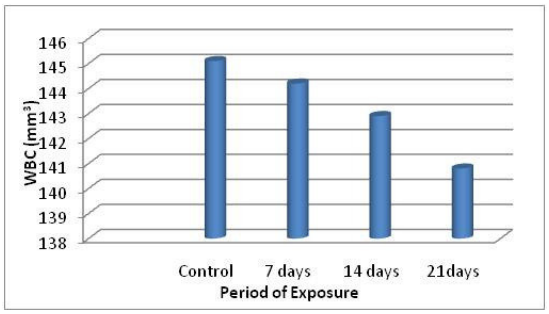


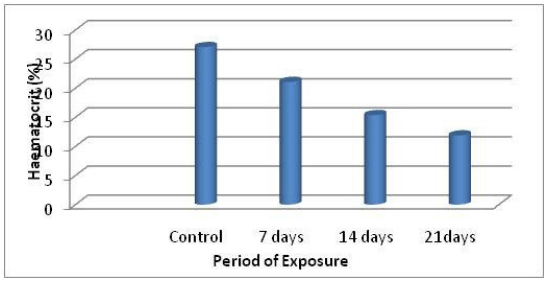



**Fig**.Haemogram of ***L. rohita***after Chronic (7 to 21 days) exposure to Polo. a) Haemoglobin content; b) Total RBC's count; c) haematocrit value; d) Mean corpuscular volume (MCV) ; e) Mean corpuscular haemoglobin (MCH); f) Mean corpuscular haemoglobin concentration (MCHC); g) Total WBC's count.

**Conclusion:** There was alteration in blood parameters of freshwater fish, *Labeorohita* after chronic exposure to pesticide Polo. MCHC concentrations were increased and all other blood parameters were decreased as compare to control. Hence an insecticide Polo produce toxic effects in freshwater fish.

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