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RESEARCH ARTICLE

HAEMATOLOGICAL ALTERATIONS IN *CYPRINUS CARPIO* AS BIOMARKERS OF CYPERMETHRIN TOXICITY

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ABSTRACT

Possible effects of a synthetic pyrethroid pesticide Cypermethrin (25%EC) were observed in a freshwater fish *Cyprinus carpio* (Linn.) Juveniles of test fish were exposed for lethal (96h LC_{50} i.e. 3.31µg/l) and sublethal ($1/10^{th}$ of 96h LC_{50} i.e. 0.331µg/l for 5, 10 and 15 days) concentrations of cypermethrin to study the haematological parameters: Red Blood Cell (RBC) count; white blood cell (WBC) count; haemoglobin (Hb); Packed Cell Volume (PCV); Mean Corpuscular Volume (MCV); mean corpuscular haemoglobin (MCH); Mean Corpuscular Haemoglobin Concentration (MCHC). RBC count, Hb content and PCV showed decrement at both lethal and sublethal concentrations. WBC count and MCHC exhibited increasing trend at sublethal and decreasing trend at lethal concentration. Elevated values of MCV were recorded at both lethal and sublethal concentrations where as MCH exhibited an increasing trend in all exposure periods of lethal and sublethal concentrations. The haematological alterations led to the conclusion that the cypermethrin has toxic effects on freshwater fish *Cyprinus carpio*, and that its presence in an aquatic ecosystem may jeopardize the health of status of the ecosystem and biota therein.

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INTRODUCTION

Among the aquatic organisms, fishes occupy an important position in the field of aquatic toxicology (Giulio and Hinton, 2008). Various stressors and pollutants generally cause rapid changes in blood characteristics of fish (Sahan *et al*., 2007). These changes can be measured and used as indicators of exposure to or effects of such toxicants, which are called biomarkers. These biomarkers enable the rapid assessment of the health of the organisms and warn about possible environmental risks associated with the toxicants. Among biological changes, haematological parameters are considered potential biomarkers of exposure to chemical agents, since the latter can induce an increase or decrease in the various haematological components (Oost *et al*., 2003). Haematological parameters have been widely used in environmental monitoring, and as indicators of disease and environmental stress (Li *et al*., 2010). Haematological parameters such as haematocrit, haemoglobin, number of erythrocytes and white blood cells are indicators of toxicity with a wide potential for application in environmental monitoring and toxicity studies in aquatic animals (Adedeji *et al*., 2000). Many studies have demonstrated changes in blood variables as a result of environmental conditions and presence of contaminants. Blood is the most important and abundant body fluid and is a vehicle for quickly mobilizing defense against trauma and diseases. Its composition often reflects the total physiological condition. The main route of entry for any pesticide is through the gills. From the gills, it is transported to various parts of the body via the blood stream. Blood provides an ideal medium for toxicity studies. Haematological variables of fish under stress are of great significance in assessing the impacts of pollutants in the biota of a particular ecosystem. Therefore, haematology has been widely used as potent bioindicator in aquatic toxicology (Sancho *et al*., 2000). Since, fishes differ considerably in their activity patterns in response to various pollutants, the blood parameters like Red blood corpuscle (RBC), White blood corpuscle (WBC), Haemoglobin (Hb), Packed cell volume (PCV), Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH) and Mean corpuscular haemoglobin concentration (MCHC) are commonly studied in fishes to assess the impact of pesticides in aquatic life.

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Fish blood can serve as a valuable tool in detecting physiological changes taking place in animal. Hence, an attempt has been made to study the effect of cypermethrin on certain blood components of *Cyprinus carpio* (Linn.) under 96h lethal (3.31µg/l for 4 days) and sublethal (0.331µg/l for 5, 10 and 15 days) concentrations of cypermethrin.

MATERIALS AND METHODS

Collection and maintenance of test organism

The freshwater fish *Cyprinus carpio* with length 6 – 8cm, weight 6.5 – 7.5g, irrespective of their sex, have been chosen as the test organisms for present investigation. Healthy and active fish were obtained from Ratna Singh Hatcheries, Kuchipudi, Guntur (A.P), India. The fish were acclimatized to the laboratory conditions in large plastic water tanks for three weeks at a room temperature of 28±1°C. Water was renewed every day with12 - 12h dark and light cycle. During the period of acclimatization, the fish were fed (*ad libitum*) with groundnut oil cake and rice bran. Feeding was stopped one day prior to the acute toxicity test. All the precautions laid by committee on toxicity tests to aquatic organisms (APHA, 1998) were followed and such acclimatized fish only were used for toxicity evaluation. If mortality exceeded 5% in any batch of fish during acclimatization, the entire batch of that fish were discarded.

Sampling of blood

Fish were euthanized by an overdose of MS-222 and then weighed and measured. Blood was sampled by caudal severance from the disease free test fish during early hours of the day and stabilized with 50 IU sodium heparin (anticoagulant)/ml blood.

Haematological examination

The haematological variables analyzed were red blood cells count (RBC), haemoglobin (Hb), white blood cells count (WBC), haematocrit (Ht), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC).

Determination of red blood corpuscles (RBC) count

RBC count was determined with an improved Neubauer crystalline counting chamber as described by (Shaperclaus, 1979). The blood was sucked up to 0.5 mark on the RBC pipette and immediately, Hayems solution as a diluent stain was drawn up to 101 mark and the pipette was rotated between the thumb and the forefinger to facilitate adequate mixing of the solution (dilution: 1:200). The counting chamber and the cover glass were cleaned thoroughly and the cover glass was placed in position over the ruled area. The fluid from the stem of the pipette was expelled as it contains only the diluting fluid. The pipette was then held at an angle of 45º with the tip of the pipette at the junction of the edge of cover glass and the counting chamber. A drop of blood was placed from the tip of the pipette on the central platform near the edge of the cover slip, so that the drop was sucked up between the central platform and the cover slip by the capillary force. The cells were allowed to settle for 2 to 3 min. The ruled area of the counting chamber was focused under the microscope and the numbers of RBC were counted in 80 small squares (4 squares of 16 at the four corners and one of 16 at the center). The cells touching the upper and left hand lines were counted. The cells touching the lower and the right hand lines were omitted.

The numbers of RBC per sq mm were calculated as follows:

The area of small square: 1/400 sq mm
The depth of the counting chamber: 1/10 mm
Therefore the volume of a small square is: 1/400 × 1/10 = 1/4000 cu mm
The dilution of blood is 1:200
Total number of RBC = n × 4000 × 200/80
n = Number of cells counted in 80 small squares

Determination of white blood corpuscles (WBC) count

WBC count was determined by following the method described Donald and Bonford (1963). The blood was drawn up to 0.5 mark of WBC pipette and immediately the diluting fluid was drawn up to the 101 mark above the bulb (the dilution fluid consists of 1.5ml of glacial acetic acid and 1 ml of aqueous gentian violet solution and made up to 100 ml with distilled water). The solution was mixed thoroughly by shaking gently and allowed to stand for 3 min. Cleaned Neubauer counting chamber and cover glass were placed over the ruled area. Excess solution was expelled and a drop of fluid was allowed to flow under the cover slip by holding the pipette at an angle of 40º and allowed to stand for 2 to 3 min. The WBC was counted in the four corner square millimeters and the number of WBC per cubic millimeter was calculated.

Estimation of haemoglobin (Hb)

Hb concentration in the blood was estimated by cyanmethaemoglobin method as described by Blaxhall and Daisley (1973). Hb is converted into cyanmethaemoglobin by the addition of potassium ferricyanide (KCN) and the colour was read in a spectrophotometer at 540 nm against a reagent blank.

Determination of packed cell volume (PCV) or haematocrit value

Packed cell volume was determined by micro haematocrit method of Schalm *et al*. (1975). The heparinised blood was filled up to the mark 100 of the haematocrit tube with the help of Pasteur pipette and centrifuged at 3000rpm for 30min. The relative volume of the height of the RBC's packed at the bottom of the haematocrit tube was recorded as packed cell volume in terms of percentage of total blood column taken in the haematocrit tube.

Determination of mean corpuscular volume (MCV)

MCV indicates the average size of the red blood cell in a given sample of blood. MCV was calculated by the following formula and expressed as femtoliter (fL).

MCV = Haematocrit (%) ×10 / RBC count

Determination of mean corpuscular haemoglobin (MCH)

MCH represents the average content of the Hb in each red blood cell. MCH is influenced by the Hb concentration and the number of RBC. MCH was calculated by the following formula and expressed in picogram (pg).

MCH = Haemoglobin(g/dL) ×10 / RBC count

Mean corpuscular haemoglobin concentration (MCHC)

MCHC reflects the average concentration of the haemoglobin in the red blood cells in a given volume of the blood. MCHC was obtained by the following formula and expressed in terms of gram percent (g%).

MCHC = Haemoglobin(g/dL) ×100 / Haematocrit (%)

RESULTS

The results of the haematological parameters are found to be time dependent. The summary of the results after exposing the fish *Cyprinus carpio* to lethal (96h) and sublethal (5, 10, and15 days) concentrations of cypermethrin is as follows. The blood parameters estimated were presented in Table 1 and Figure 1.

Red blood corpuscle (RBC) count

Results of total erythrocyte count showed alterations at both lethal and sublethal concentrations of cypermethrin. The RBC count was 2.98 million/cu.mm in the blood of control fish and this count was decreased to 1.32 millions/cu.mm at lethal concentration at 96h of exposure and 1.54 millions/cu.mm at sublethal concentration at 15 days exposure period. The decreasing trend was the function of exposure period and time. The percentage change in the RBC count was also calculated.

Table 1. Haematological alterations in *Cyprinus carpio* on exposure to lethal and sublethal concentrations of cypermethrin

Parameters	Control	Lethal (96h)	Sublethal		
			Day 5	Day 10	Day 15
RBC (Millions/cu.mm)	02.98 ± 0.18	01.32 ± 0.14 (55.70)	02.15 ± 0.28 (27.85)	01.93 ± 0.21 (35.23)	01.54 ± 0.31 (48.32)
WBC (Cells/cu.mm)	11.64 ± 0.15	07.12 ± 0.13 (38.83)	12.18 ± 0.20 (04.63)	14.01 ± 0.19 (20.36)	14.73 ± 0.43 (26.54)
HB (g/dl)	07.06 ± 0.64	2.54 ± 0.27 (64.02)	06.91 ± 0.59 (02.12)	06.05 ± 0.61 (14.28)	06.24 ± 0.38 (11.61)
PCV (%)	27.18 ± 0.34	11.94 ± 0.52 (56.07)	24.12 ± 0.48 (11.25)	21.61 ± 0.25 (20.49)	20.75 ± 0.49 (23.65)
MCV (cu µm)	62.19 ± 0.22	80.55 ± 0.37 (29.52)	66.33 ± 0.17 (06.65)	71.43 ± 0.15 (14.85)	65.05 ± 0.27 (04.59)
MCH (pg)	20.54 ± 0.14	25.82 ± 0.57 (25.70)	23.65 ± 0.28 (15.14)	25.05 ± 0.41 (21.95)	24.33 ± 0.12 (18.45)
MCHC (%)	28.32 ± 0.46	28.16 ± 0.23 (0.56)	30.31 ± 0.16 (7.02)	30.02 ± 0.25 (6.00)	31.50 ± 0.32 (11.22)

Values are the mean of 5 observations
Standard Deviation is indicated as (±)
Values are significant at $p < 0.05$
Percent changes over control are given in Parenthesis

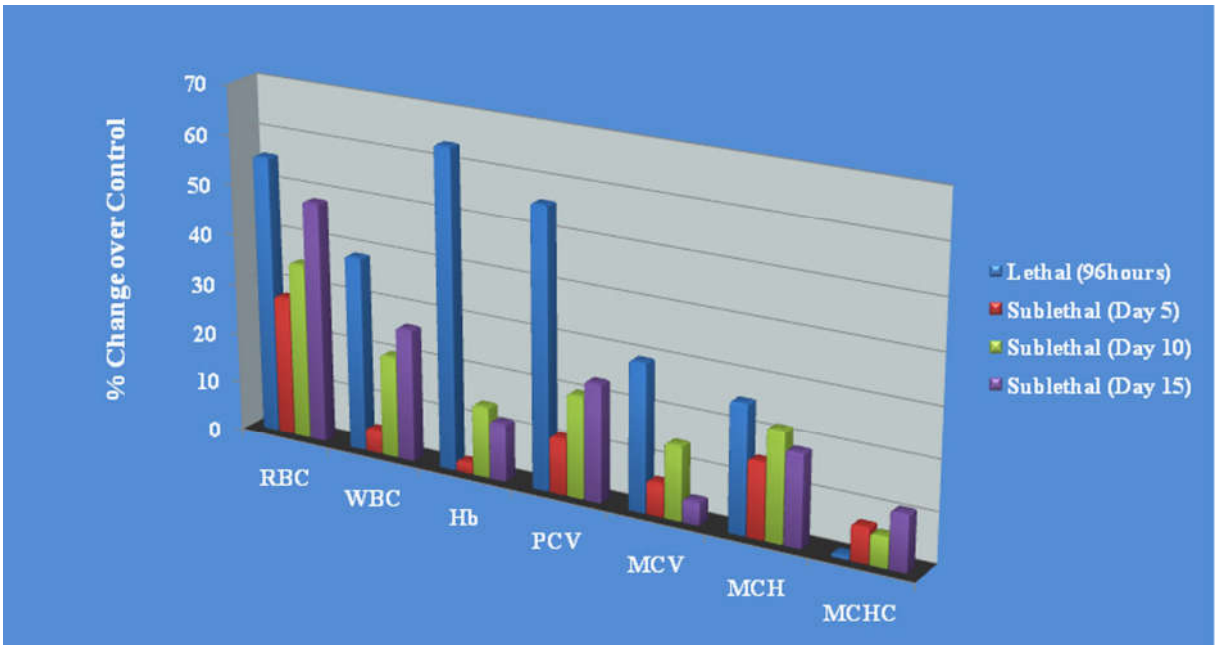

Figure 1. Haematological alterations in *Cyprinus carpio* exposed to lethal and sublethal concentrations of Cypermethrin 25% EC

At lethal concentration maximum reduction of 55.70% was observed at 96h exposure period. At sublethal concentration increase in the reduction was observed up to day 10 (35.23%) and the reduction was continued up to day 15 (48.32%).

White blood corpuscle (WBC) count

The WBC count altered specifically and the WBC count exhibited a different trend when compared to the RBC count. It exhibited a slight increasing trend as the increasing number of days at sublethal concentrations as the increasing number of days. The increasing trend was a function of exposure periods. At lethal concentration for 96h exposure period slight decrease was observed. Maximum count 38.83% was observed at 96h under lethal concentration. In sublethal concentration of cypermethrin, WBC count increased continuously from day 5 (4.63%) to day 10 (24.33%) and day 15 (26.54%).

Haemoglobin (Hb) content

The haemoglobin content of blood of *Cyprinus carpio* was 7.06±0.64 in control fish. The Hb content decreased in test fish when exposed to both lethal and sublethal concentrations. The decrease was 64.02% at 96h exposure period. At sublethal concentration, decrement was intensified from day 5 (2.12%) to day 10 (14.28%) and at 15 days (11.61%) reduction was registered over control. The decreasing trend in Hb content is comparable to that of RBC count in the blood of fish exposed to different durations.

Packed cell volume (PCV)

The trend of PCV value was like that of RBC and Hb at lethal and sublethal concentration. In the lethal concentration decrease was up to 96h (56.07%). During sublethal concentration PCV was decreased from day 5 (11.25%) to day 10 (20.49%). While approaching towards 15th day, decrease was further intensified up to 23.65% over control.

Mean corpuscular volume (MCV)

MCV value calculated on the basis of PCV and RBC values. Elevated values of MCV were recorded over control (62.19±0.22) at both lethal and sublethal concentrations. At lethal concentration, 29.52% elevation was observed at 96h exposure. In contrary at sublethal concentration the elevation was seen up to day 5 (6. 65%), day 10 (14.85%) and which later decreased at day 15 (4.59%) over earlier periods of exposure.

Mean corpuscular haemoglobin (MCH)

MCH exhibited increase in all exposure periods of lethal concentration. Maximum increase was observed at 96h (25.70%). At sublethal concentration, the increase was 15.14%, 21.95% and 18.45% at 5, 10 and 15 days respectively.

Mean corpuscular haemoglobin concentration (MCHC)

The MCHC of blood of *Cyprinus carpio* was 28.32±0.46 in control fish. The MCHC value decreased in lethal and increased at sublethal concentrations. The decrease was 0.56% at 96h exposure period. At sublethal concentration, maximum increment was observed at day 15 (11.22%) and minimum was at day 5 (7.02%) over control.

DISCUSSION AND CONCLUSION

In the present investigation, *Cyprinus carpio* exposed to cypermethrin presented a significant decrease in total erythrocyte count, haemoglobin percentage, PCV values and an increase in WBC, MCV, MCH and MCHC over control. These results express a probable condition of anemia, since it is known that many chemical pollutants, including pesticides can induce anemia in fish (Min and Kang, 2008). This anemia may be due to ROS-induced oxidative injury via oxidation of haemoglobin or other cellular components (Bloom and Brandt, 2008). The reduction in RBC count either by haemolysis or erythropoietic disorders and also the reduction in Hb contents by haemopoietic disorders lead to anaemic condition in fish. The anaemia may affect the general well being of the fish.

Anaemic condition in fish is a consequence of inductive haemolytic effects on exposure to cypermethrin, due its high affinity to membrane phospholipids, which accounts for its lytic activity. In addition cypermethrin, with a slightly higher lipophilicity and is known to partition extensively into the phospholipid component of biological membranes. Hence it is suggested, that anaemia found in cypermethrin exposed fish in the present study is a consequence of an interaction between cypermethrin and the erythrocyte membrane. The reduction in RBC, Hb and Hct in test fish can be interpreted as a compensatory response that reduce the oxygen carrying capacity to maintain gas transfers and indicates a change in the water blood barrier for as exchange in the gill lamellae (Jee *et al*., 2005). The results indicate that cypermethrin causes haematological changes that can interfere in oxygen uptake, which may jeopardize the animal's overall health. The results are in accordance with earlier reports stated that a significant decrease in RBC's, haemoglobin and packed cell volume of freshwater fish exposed to cypermethrin (Kannan *et al*., 2014; Akinrotimi *et al*., 2012; Velisek *et al*., 2011).

The increase in WBC is considered as an adaptive mechanism. This may be due to the direct stimulation of the immunological defense mechanism against stress (Henry *et al*., 1978) which may be associated with the tissues damage. Such increase in WBC count may be due to lymphocytosis and immune response in cypermethrin exposed fish (Agarwal and Srivastava, 1980). Decrease in WBC count at lethal concentration might be due to series of changes in the immunological set up of the fish under pesticide stress (Anandakumar, 1994). Kalavathy *et al.* (2001) suggested that decrease in WBC count could be because of autolysis, caused due to haemolytic enzymes leaked out by cells on exposure to cypermethrin. Increase in the number of WBC (leucocytes) of treated fish under sublethal concentrations reflects a general state of toxaemia exhibiting impairment of the defense mechanism, and is manifested into leucocytosis to cope with such a situation. Similar results were reported in *Catla catla* (Vani *et al*., 2012), *Labeo rohita* (Adhikari *et al*., 2004), *Channa orientalis* (Shinde *et al*., 2014), *Cyprinus carpio* (Masud and Singh, 2013), *Clarias gariepinus* (Akinrotimi

et al., 2012). Since the leucocyte cells are important cells in the immune system play a major role in the defense mechanism of the fish. Thus increasing or decreasing numbers of leucocyte cells are a normal reaction to a chemical such as cypermethrin as in the present investigation. Declining trend in packed cell volume or haematocrit values may be due to impaired oxygen supply to various tissues, resulting in slow metabolic rate and low energy production. The decline in PCV might shrink cell size due to intoxication. Similar results were noted in *Channa punctatus* following cypermethrin treatment (Saxena and Seth, 2002). Recently, Khatun *et al*. (2014) also opined that the decrease in the PCV of *Cyprinus carpio* exposed sublethal concentration of cypermethrin was the result of either rapid oxidation of haemoglobin to methaemoglobin or release of oxygen radical brought about by toxic stress of the pesticide. According to Adedeji, 2009, decrease in PCV in the test fish could be ascribed to reduction in erythrocyte lifespan and/or a suppressive effect of the active substance of the pesticide on the erythropoietic tissues, resulting in the failure of erythrocyte production. Increased MCV may be caused due to endosmosis which leads to the passage of solvent from less concentrated solution to more concentrated one. This results in haemodilution, further increasing the MCV value as suggested by Anandkumar (1994).

As MCH and MCHC are derived from Hb and RBC, any sort of alteration in the levels of Hb and RBC would result in the alteration of MCH and MCHC. Elevated levels of MCH and MCHC recorded in the present investigation were in accordance with the findings of Parma *et al*. (2007) and Atamanalp *et al*. (2002). Cypermethrin induced haematological alterations were studied by Kannan *et al*., 2014, Vani *et al*., 2012 (*Catla catla*); Khatun *et al*., 2014, Vani *et al*., 2012 and Adhikari *et al*., 2004 (*Labeo rohita*); Shinde *et al*., 2014 (*Channa orientalis*); Masud and Singh, 2013 and Velisek *et al*., 2011, Dobsikova *et al*., 2006, Dorucu and Girgin, 2001 (*Cyprinus carpio*); Akinrotimi *et al.,* 2012 (*Clarias gariepinus*); Chandra Lekha and Dutta, 2012 (*Heteropneustes fossilis*); Velisek *et al*., 2011 and 2006, Cakmak and Girgin, 2003 and Atamanalp *et al*., 2002b (*Onchorhynchus mykiss*); Borges *et al*., 2007 (*Rhamdia quelen*); Parma *et al*., 2007 (*Prochilodus lineatus*) and Saxena and Seth, 2002 (*Channa punctatus*).

Kannan *et al*. (2014) investigated the effects of cypermethrin (10% EC) in the concentration of 0.0006ml/l on *Catla catla* for 24h and reported significant (P<0.01) increase in the number of erythrocytes, segmented neutrophil granulocytes, and a significant (P<0.01) decrease in mean erythrocyte volume, mean erythrocyte haemoglobin and lymphocyte count. Khatun *et al*. (2014) studied the effect of cypermethrin on haematology of *Labeo rohita* exposed to two concentrations of cypermethrin (0.15 and 0.30µl/l) for 96h. The blood parameters viz., total WBC and RBC count, Hb, PCV, MCV, MCH and MCHC values were analyzed. A decrease in WBC and RBC count, Hb and PCV values were found in both concentrations of cypermethrin exposure (P<0.05). MCV value was decreased in low concentration and increased in high concentration whereas MCH and MCHC values were increased in low concentration and decreased in high concentration of cypermethrin exposure as compared to the control fish (P<0.05). Shinde *et al*., 2014 observed remarkable decrease in total RBC in *Channa orientalis* exposed to cypermethrin. Masud and Singh (2013) assessed impact of short term exposures of cypermethrin safe concentrations (1/2 (0.05µl/l) and 1/10 (0.01µl/l) parts of safe concentration) on some haematological parameters. The haematological analysis showed significant reduction in red blood cells (RBCs) count and haemoglobin (Hb) value while total white blood cells (WBCs) count were significantly increased in the fish *Cyprinus carpio.* Complete recovery was obtained in all the parameters after a recovery period of 7 days.

Akinrotimi *et al*. (2012) observed juveniles of *Clarias gariepinus* exposed to 0.05, 0.10, 0.20 and 0.25ppm cypermethrin solution for 10 days showed a significant (P<0.05) reduction which is concentration dependent in the values of PCV, RBC, Hb, Leucrocrit, Lymphocytes, eosinophils, monocytes, Thrombocytes, MCHC, MCH and MCV while the values of WBC, neutrophils, and ESR, increased significantly (P<0.05). These alterations were more pronounced at 0.25ppm.

Chandra Lekha and Dutta (2012) observed haematological changes in *Heteropneustes fossilis* viz. total erythrocyte count (TEC) and haemoglobin content (Hb). Fish subjected to 0.1µg/l ($1/6^{th}$ of 96h LC_{50}) of cypermethrin 10% EC for 24, 48, 72 and 96h showed significant decrease (p<0.05) in total erythrocyte count (TEC) and haemoglobin content (Hb). The most common blood parameters monitored include total erythrocyte count, total leucocyte count, and haemoglobin and haematocrit content, which were found to decrease on cypermethrin exposure in the Indian major carps *Labeo rohita* and *Catla catla* (Vani *et al*., 2012). Yasser, 2012 observed haematological and micronuclei alterations in *Cyprinus carpio* exposed to cypermethrin. Fish showed a significant decrease (p<0.05) in erythrocytes count (EC), haemoglobin percentage (Hb), haematocrit counts (HCT) as well as mean cellular haemoglobin concentration (MCHC) and a significant increase (p<0.05) values of total leucocytes (TLC) and blood indice mean cellular volume (MCV) compared to the control group with respect to the increase in exposure in both sublethal concentrations. There was no significant difference in value of mean cellular haemoglobin (MCH) in the experimental fish compared with control.

Rainbow trout exposed to cypermethrin exhibited significantly lower (*P*<0.05) numbers of developmental forms of myeloid sequence and segmented neutrophilic granulocytes than did untreated fish. Moreover, cypermethrin exposure in common carp resulted in significantly (*P*<0.01) higher values of RBC, MCV, MCH, and lymphocyte count (*P*<0.01) compared to controls (Velisek *et al*., 2011). Borges *et al*., 2007 recorded increased lymphocyte, leucocyte, and erythrocyte counts, packed cell volume, and haemoglobin in *Rhamdia quelen* challenged with cypermethrin. *Prochilodus lineatus* exposed to sublethal concentrations of cypermethrin (0.3 and 0.6mg/l) for 2, 5 and 8 days showed that with the increase of exposure time total erythrocyte (RBC), haemoglobin (Hb), haematocrit (Ht) and mean corpuscular haemoglobin concentration (MCHC) values decreased but mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) values increased (Parma *et al*., 2007). Dobsikova *et al*., 2006 reported that

Cyprinus carpio exposed Alimetrin 10EM in the concentration of 29.1µg/l corresponding to 29.1µg/l of cypermethrin for 96h showed a significant ($P<0.01$) increase in the number of erythrocytes, segmented neutrophile granulocytes, developmental forms of myeloid sequence and eosinophiles, and a significant ($P<0.01$) decrease in mean erythrocyte volume, mean erythrocyte haemoglobin and lymphocyte count. Velisek *et al*. (2006) found that the acute exposure to cypermethrin resulted in a significant decrease in count of developmental forms of myeloid sequence and the segmented neutrophilic granulocytes. Effects of cypermethrin on haematological parameters and their complete recovery were studied in *Labeo rohita* as a function of exposure time by Adhikari *et al*. (2004). Exposure to sublethal levels of cypermethrin resulted in significantly ($P<0.05$) lower values for erythrocyte count (RBC), haemoglobin content (Hb), and haematocrit compared with the control group. In contrast, there was a significant increase ($P<0.05$) in leukocyte count (TLC) in the pesticide-treated group. Mean cell volume (MCV) and mean cell haemoglobin (MCH) increased in response to cypermethrin exposure.

Das and Mukherjee (2003) reported a reduction in haemoglobin levels with no associated changes in red blood cell count and an increase in the total leukocyte count in *Labeo rohita* exposed to cypermethrin. According to Çakmak and Girgin (2003), cypermethrin have paramount effects on different haematological parameters such as levels of haematocrit, haemoglobin, leukocyte, RBC and mean corpuscular haemoglobin concentration etc. of rainbow trout (*Oncorhynchus mykiss,* Walbaum). Haematocrit levels, haemoglobin, leucocyte, RBC and MCHC decreased with increasing concentrations ($P<0.01$). MCV level increased ($P<0.01$) and MCH level was not affected (($P>0.05$). Atamanalp *et al.* (2002) found a significant increase ($P<0.05$) in the levels of RBC and a significant decrease ($P<0.05$) in the Hb, MCH, MCHC, thrombocyte count and erythrocyte sedimentation rate in rainbow trout following cypermethrin acute exposure. Saxena and Seth (2002) reported haematological aspects of *Channa punctatus* exposed to 0.1, 0.2, 0.35 and 0.5ppm of cypermethrin for 5, 10, 15, 20, 25, and 30 days. RBC count decreased abruptly on day 5 and declined further with the increase in concentration. A significant decrease was observed in HB percentage, PCV and protein. Average density of blood was also showed little change.

A decrease in the levels of PVC, Hb, LEU, and RBC was reported in *Cyprinus carpio* after poisoning with cypermethrin (Dorucu and Girgin, 2001). The present study suggested that the perturbations in blood indices attributed to a defense reaction against toxicity of cypermethrin through the stimulation of erythropoesis or may be due to the disturbances that occurred in both metabolic and haemopoetic activities of fish exposed to below safe concentrations of cypermethrin. The toxicant caused haematological disturbance which could lead to impairment of the fish ability to combat diseases, reduce its chances for survival and potential for growth and reproduction. Thus, cypermethrin in the aquatic medium is a major factor responsible for drastic changes in the fish blood. However, it would be more rational to mention that alterations in haematological parameters in pesticide exposed fish will provide important information on the general well being of fish. Thus, fish blood following exposure to pesticides is the best suitable biomarker in the field of aquatic toxicology.

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