RESEARCH ARTICLE

Haematotoxicity of Cypermethrin (25% EC) to white carp (*Cirrhinus mrigala*)

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ABSTRACT

Cyperkill 25%EC is a synthetic pyrethroid pesticide widely used in Guntur district for pest control in agri ventures due to its effective control against different pest species in a definitive and constant way with low cost. Its presence in freshwater environs is very common in this area. Contamination of aquatic ecosystems with this toxicant affects all groups of aquatic fauna including fish which are non target biota. A static-renewal bioassay was conducted to assess the acute and sublethal toxicity of cypermethrin on some haematological parameters of white carp (*Cirrhinus mrigala*). Juveniles of experimental fish were exposed for lethal (96 h LC_{50} i.e. 4.23µg/L) and sublethal ($1/10^{th}$ of 96 h LC_{50} i.e. 0.423µg/L for 5, 10 and 15 days) concentrations of cypermethrin. Decreased tendency at both lethal and sublethal concentrations was evident in RBC count, Hb content and PCV. At the same time an increasing trend in WBC count and MCHC at sublethal and decreasing trend at lethal concentration. MCV and MCH values were elevated at both lethal and sublethal concentrations. Toxicity derived alterations observed in haematological indices during this study led to a conclusion that the cypermethrin has detrimental effect on the test fish and its presence in an aquatic environment may severely threaten the health of the ecosystem and its living component.

Keywords: Cyperkill, Haematological indices, Toxicity, Bioassay, Synthetic pyrethroids.

INTRODUCTION

Industrial effluents, domestic sewage and pesticides are the important sources of aquatic pollution. Aquatic bodies that run through agricultural or industrial areas have high vulnerability of being polluted by surface runoff and leaching by a variety of pesticides. Aquatic ecosystems, greater part of natural environment are continuously being polluted with a wide variety of environmental pollutants such as pesticides from industrial, agricultural and domestic activities (Okuku and Peter, 2012). They are facing the threat of shrinking genetic base and biodiversity due

to indiscriminate use of these toxic chemicals (Rahman *et al*., 2002) which tend to accumulate in organisms and are persistent because of their chemical stability (Wepener *et al*., 2001). Pesticides are one of the most potentially harmful chemicals introduced into the environment. Though they have contributed considerably to human welfare, their adverse effects on non-target organisms are significant (Ullah *et al*., 2015). Pesticides in water may induce severe ecological consequences, generating reorganizations of the biocenosis, changing it and consequently affecting aquatic ecosystems integrity (Vosyliene and Jankaite, 2006). Long term exposure to these toxic chemicals causes countless abnormalities and reduces the life span of organisms (Naz *et al*., 2011). There is an increasing concern world over on the indiscriminate use of such chemicals that result in environmental pollution and toxicity risk to non-target species (Velisek *et al*., 2007). Pyrethroids are preferred above organophosphates, carbamates and organochlorines as these have high efficiency, low toxicity and easy biodegradability (Sharaf *et al*., 2010). Although synthetic pyrethroids have been claimed as safe and environmentally friendly because of their selective toxicity to insects, low persistence and low toxicity to mammals and birds, they are highly toxic to a number of other non-target organisms including fish, lobster, shrimp, mayfly nymphs and many species of zooplankton (Oudou *et al*., 2004).

Blood is a unique medium in which the initial processes taking place in an organism are reflected. The observation on different haematological parameters provide a good deal in diagnosing the effect of environmental stress on an animal and in fact would give an insight into changes induced in the circulating fluid. Study of haematological parameters can indicate physiological response to a contaminated environment (Dethloft *et al*., 2001). Haematological parameters such as hematocrit, hemoglobin, 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). The study of the fish blood parameters are important for determining factors related to its physiological capacity (Wells *et al*., 2005). Many studies have demonstrated changes in blood variables as a result of environmental conditions and presence of contaminants. Comprehensive ecotoxicological studies on different fish species to locally or regionally used toxicant would not only help us to protect the environment as a whole, but also provide ecotoxicological data which can be used for the ecomanagement of such chemicals in the region or elsewhere. Hence in the present study, toxicity of cypermethrin (a type II synthetic pyrethroid) was studied on certain haematological parameters of a commonly available and highly consumed fish *Cirrhinus mrigala*.

MATERIALS AND METHODS

Fingerlings of the experimental fish with length 6 – 7cm, weight 7 – 7.5g, irrespective of their sex 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 the experiment. If mortality exceeded 5% in any batch of fish during acclimatization, the entire batch of that fish were discarded.

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. RBC count was determined with an improved Neubauer crystalline counting chamber as described by (Shaperclaus, 1979). WBC count was determined by Donald and Bonford (1963) method. Hb concentration was estimated by cyanomethaemoglobin method (Blaxhall and Daisley, 1973). Packed cell volume was determined by micro haematocrit method (Schalm *et al*., 1975). MCV was calculated by using a formula (MCV = Haematocrit (%) ×10 / RBC count) and expressed as femtoliter. MCH was calculated by a formula (MCH = Haemoglobin(g/dL)×10/RBC count) and expressed in picogram. MCHC was obtained by using a formula [MCHC = Haemoglobin (g/dL) ×100 / Haematocrit (%)] and expressed in terms of gram percent (g%). The data were subjected to standard statistical analysis. The values reported are mean ± standard error of mean of 5 observations. One way

ANOVA and Duncan multiple range tests were used for group wise comparisons.

RESULTS

Haematological parameters observed after exposing the fish *Cirrhinus mrigala* to lethal (96h) and sublethal (5, 10, and15 days) concentrations of cypermethrin were presented in table 1. Alteration in total erythrocyte count was observed at both lethal and sublethal concentrations. A decreased trend was noticed in RBC count from control (2.94 million/cu.mm) to the test fish (1.30 and 1.55 million/cu.mm) at 96h lethal and 15 days sublethal exposure periods respectively and the trend was the function of exposure period and time. A maximum reduction of TEC (55.78%) was observed at 96h lethal concentration and the increase in the reduction was observed up to day 10 (34.69%) and was continued up to day 15 (47.27%) at sublethal concentrations. When compared to TEC, WBC count exhibited a different trend by showing a slight increase as the increasing number of days at sublethal concentrations and a slight decrease at 96h lethal. A maximum percent (38.75%) of decrease at 96h lethal and a continuous increment from day 5 (4.65%) to day 10 (20.84%) and day 15 (26.70%) were noticed at sublethal concentrations. There was a decreasing trend in Hb content from the control fish (7.03±0.34 g/dl**)** which comparable to that of TEC at both lethal and sublethal concentrations and durations. The decrease was 64.15% at 96h lethal and it was intensified from day 5 (1.84%) to day 10 (14.22%) and at 15 days (11.52%) over control at sublethal concentrations. Decreasing trend was observed in PCV like that of RBC and Hb at lethal and sublethal concentrations. In lethal, decrease was up to 96 h (56.09%) and at sublethal concentrations, PCV was decreased from day 5 (11.23%) to day 10 (20.36%). While approaching towards 15th day, decrease was further intensified up to 23.68% over control. MCV values were elevated over control (62.17±0.42) at both lethal and sublethal concentrations. 29.53% of elevation was observed at 96h lethal and the elevation was seen up to day 5 (6. 65%), day 10 (14.92%) and which later decreased at day 15 (4.6%) over earlier periods of exposure at sublethal concentrations. There was an increment in MCH over control in all exposure periods at both lethal and sublethal concentrations. Maximum increase was observed at 96h (25.79%). At sublethal concentration, the increase was 15.16%, 21.98% and 18.72% at 5, 10 and 15 days respectively. MCHC showed an increment at sublethal and decrement at lethal concentrations. The decrease was 0.6% at 96h exposure period. At sublethal concentration, maximum increment was observed at day 15 (11.37%) and minimum was at day 5 (7.06%) over control (28.30±0.49).

Table 1: Haematological alterations in *Cirrhinus mrigala* on exposure to lethal and sublethal concentrations of cypermethrin

Parameters	Control	Lethal (96h)	Sublethal		
			Day 5	Day 10	Day 15
RBC (Millions/cu.mm)	02.94 ± 0.15	01.30 ± 0.18 (55.78)	02.11 ± 0.23 (28.23)	01.92 ± 0.28 (34.69)	01.55 ± 0.33 (47.27)
WBC (Cells/cu.mm)	11.61 ± 0.21	07.11 ± 0.11 (38.75)	12.15 ± 0.29 (04.65)	14.03 ± 0.17 (20.84)	14.71 ± 0.46 (26.70)
HB (g/dl)	07.03 ± 0.34	2.52 ± 0.25 (64.15)	06.90 ± 0.59 (01.84)	06.03 ± 0.64 (14.22)	06.22 ± 0.35 (11.52)
PCV (%)	27.15 ± 0.52	11.92 ± 0.43 (56.09)	24.10 ± 0.43 (11.23)	21.62 ± 0.23 (20.36)	20.72 ± 0.44 (23.68)
MCV (cu µm)	62.17 ± 0.42	80.53 ± 0.17 (29.53)	66.31 ± 0.14 (06.65)	71.45 ± 0.19 (14.92)	65.03 ± 0.21 (04.60)
MCH (pg)	20.51 ± 0.17	25.80 ± 0.35 (25.79)	23.62 ± 0.27 (15.16)	25.02 ± 0.61 (21.98)	24.35 ± 0.10 (18.72)
MCHC (%)	28.30 ± 0.49	28.13 ± 0.26 (0.600)	30.30 ± 0.14 (7.06)	30.05 ± 0.20 (6.18)	31.52 ± 0.38 (11.37)

Values are the mean of 5 observations
Standard Deviation is indicated as (±)
Values are significant at p < 0.05

DISCUSSION

The evaluation of haematological characteristics of blood in fish has become an important means to understanding normal and pathological processes and toxicological impacts (Suvetha *et al*., 2010; Velisek *et al*., 2010). Fish blood is a pathophysiological reflector of its whole body and therefore, its haematological parameters are important in diagnosing altered structural and functional status of the fish exposed to various toxic chemicals (Banaee *et al*., 2011, Adhikari *et al*., 2004, Saxena and Seth, 2002). In the present investigation, *Cirrhinus mrigala* exposed to cypermethrin showed a significant decrease in total erythrocyte count, haemoglobin percentage, PCV values and an increase in WBC, MCV, MCH and MCHC over control fish. The reduction in TEC and Hb are observed in the present study was accompanied by a decrease in PCV which demonstrated the physiological dysfunction of the hemopoietic system in test fish. It has been shown that the erythrocyte number and haemoglobin level may vary with oxygen requirements (Tavares *et al*., 2004).

The reduction of RBC is mainly due to development of hypoxic condition during the treatment which inturn leads to increase in destruction of RBC or decrease in rate of formation of RBC due to non availability of Hb content in cellular medium (Chen *et al*., 2004). Decreased trend in RBC count observed in the present study was either by haemolysis or erythropoietic disorders and the decrement in Hb content by haemopoietic disorders lead to anaemic condition in test fish. The anaemic condition in fishes is attributed to an inhibition on erythrocyte production or haemodilution (Larson, 1975). The anaemia may affect the general well being of the fishes. Pesticide induced anemia in test fish may be due to the inhibitory effect of the toxic substance on the enzyme system responsible for the synthesis of haemoglobin. It may also be due to impaired intestinal absorption of iron, as suggested by Joshi *et al*. (2002). Decreased trend in RBC, Hb and PCV in the present study is in accordance with earlier investigations conducted by various researchers on different fish species (Neelima *et al*., 2015; Khatun *et al*., 2014; Akinrotimi *et al*., 2012). Decreased values in RBC, Hb and PCV can be interpreted as a compensatory response that reduce the oxygen carrying capacity of the blood to maintain gaseous exchange and indicates a change in the water blood barrier for gas exchange in the gill lamellae (Jee *et al*., 2005).

It is known that WBC cells are normally lower in healthy fish and could be used as a significant indicator to toxic stress (Christensen *et al*., 1978). They are important cells in the immune system which play a major role in the defense mechanism of the fish during infestation by stimulating the haemopoietic tissues and the immune system by producing antibodies and chemical substances working as defense against infestation (Hassen, 2002), a change in its medium due to xenobiotic transformation. Thus increasing numbers of leucocytes is a normal reaction to the toxicant in the present study. Increase in WBC count in the present investigation is suggestive of an increase in antibody production for survival of the fish exposed to the toxicant cypermethrin which was in accordance with Joshi *et al*., 2002.

Increased WBC count indicate hypersensitivity of immune cell resulting into immunological reactions to produce antibodies to cope up with the stress induced by cypermethrin (Ramesh and Saravanan, 2008). Enhancement in WBC count in test fish during toxic exposure period of cypermethrin is considered as an adaptive mechanism to overcome the toxic stress which was in accordance with the previous studies (Masud and Singh, 2013; Borges *et al*., 2007). Increment in WBC count may be due to the direct stimulation of the immunological defense mechanism against the toxicant. Such increase in WBC count may be due to lymphocytosis and immune response in exposed fish (Ates *et al*., 2008). Such lymphocyte response might be due to the presence of toxic substances may be associated with pollutant induced tissue damage and severe disturbance of the non-specific immune system leading to increased production of leucocytes (Das and Mukherjee, 2003).

Significant decrease was observed in Hb levels at both lethal and sublethal concentrations which may impair oxygen supply to the fish tissues, thus resulting in a slow metabolic rate and low energy production as suggested by Ahmad *et al*., 1995. The significant decrease in the haemoglobin concentrations may also be due to either an increase in the rate at which the haemoglobin is destroyed or to a decrease in the rate of haemoglobin synthesis (Reddy and Bashanihideen, 1989; Reddy *et al*., 1989). The decrease in haemoglobin concentration signifies that the fish's

ability to provide required oxygen levels to the tissues is restricted considerably and will result in decrease of physical activity (Wepener *et al*., 1992). Buckley *et al*. (1976) reported that prolonged reduction in haemoglobin content is deleterious to oxygen transport and any blood dyscrasia and degeneration of the erythrocytes could be ascribed as pathological conditions in fishes exposed to toxicants.

Decrease in PCV of blood in the present study is in accordance with the decrease in RBC count. The similar results were obtained in *Catla catla* (Vani *et al*., 2012), *Channa punctatus* (Saxena and Seth, 2002), *Clarias gariepinus* (Akinrotimi *et al*., 2012), *Cyprinus carpio* (Neelima *et al*., 2015; Yasser, 2012; Dorrucu and Girgin, 2001), *Labeo rohita* (Khatun *et al*., 2014; Vani *et al*., 2012; Adhikari *et al*., 2004), *Oncorhynchus mykiss* (Cakmak and Girgin, 2003) and *Prochilodus lineatus* (Parma *et al*., 2007). Significant decreases in the haematocrit values recorded after exposure to cypermethrin are indicative of anaemia and haemodilution possibly due to gill damage or/and impaired osmoregulation (Larsson *et al*., 1985).

The perturbations in MCV, MCH and MCHC in the present study may be attributed to a defense against the toxicity of cypermethrin through the stimulation of erythropioesis or may be related to the decrease in RBCs, Hb and Hct due to exaggerated disturbances that occurred in both metabolic and haemopoietic activities of fish exposed to lethal and sublethal concentrations of pollutants (Mousa, 1999). Fish in toxic media showed increased MCV and MCH as a response to overcome hypoxic conditions as reported by Rauf and Arain, 2013. The increase in MCV and MCH values indicates that a reduced RBC count may be due to the destruction of erythrocytes or their decreased synthesis in bone marrow (Morgan *et al*., 1980).

CONCLUSION

Haematological parameters in fish are very sensitive to environmental changes. The present study revealed that cypermethrin has profound influence on the haematological parameters of the test fish, *Cirrhinus mrigala*. Hence, from the present study the toxic effect of cypermethrin is obvious by the significant changes in various haematological indices of the blood. Alterations in the blood parameters may provide the early warning signs of pollution caused by various xenobiotics. The findings of present study will provide more effective understanding of toxicological consequences of pesticides such as cypermethrin and its safer levels in the aquatic medium to safeguard aquatic environments.

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