Indian J. Anim. Hlth. (2017), 56(2):187-196

Research Article

EFFECTS OF CYPERMETHRIN ON HAEMATOLOGICAL PARAMETERS OF AIR BREATHING FISH *Heteropneustes fossilis* AND USE OF DIETARY ASCORBIC ACID AS AN ANTIDOTE

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Histopathological changes in erythrocytes and changes in haematological profile were observed in adult *Heteropneustes fossilis* exposed to cypermethrin respectively to lethal dose (1.0 µg / L) for 24h and sub-lethal doses (0.3 and 0.5 µg / L) for 72h. The two sub-lethal doses were tested alone as well as with low (50 mg/100g) and high (100 mg/100g) level of dietary ascorbic acid (AA). The results showed that the lethal dose of cypermethrin resulted in complete dissolution of plasma membrane and the cytoplasm thereby exposing the nucleus. Under the sublethal doses of cypermethrin total erythrocyte count (TEC), packed cell volume (PCV), mean cell (corpuscular) volume (MCV), haemoglobin (Hb), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC) decreased in fish fed diet with no supplement of AA as compared to control. These effects of cypermethrin were counteracted by high level of dietary AA, while low the level of dietary AA level failed to counteract the effects of cypermethrin. It is concluded from this study that dietary supplement of ascorbic acid @ 100mg/100g is capable to prevent adverse effects of cypermethrin on haematological parameters of *H. fossilis*.

Key words: Ascorbic acid, Cypermethrin, Fish, Haemoglobin, *Heteropneustes fossilis*

Use of synthetic pesticides in the agricultural fields to control insect pests, is a key factor behind sharp increase in crop production and stock of surplus food grain in India during the last two decades. However, continued use of the conventional

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pesticides is a cause of concern for environment and human health, because many of these pesticides are persistent in environment, highly toxic to aquatic and terrestrial animals and have the potential to bio magnify through food chain. Because of these concerns a new class of pesticides, the synthetic pyrethroids have developed to replace the conventional pesticides throughout the world. The pyrethroids are photo degradable and are lowly toxic to birds and mammals (Moore and Waring, 2001). Several pyrethroids have developed since last few years and these are now widely used in the agricultural fields to control insect pests (Parashar *et al*., 2001 and Wardhaugh, 2005).

Cypermethrin is a modern pyrethroid pesticide, which is extensively used in India to control insect pests of agricultural fields (Kaviraj and Gupta, 2014). Unlike birds and mammals, cypermethrin is highly toxic to fish 96h LC_{50} being 0.67 µg/L for *Heteropneustes fossilis* (Saha and Kaviraj, 2003), 2.60 µg/L for *Cyprinus carpio* (Saha and Kaviraj, 2008) and 2.0 µg/L for Atlantic salmon *Salmo salar* (Smith and Stratton, 1986). The embryos (48 h LC_{50}- 0.909 µg / L) and larvae (96 h LC_{50}- 0.809 µg / L) of common carp are even more sensitive to cypermethrin (Aydin *et al.*, 2005).

Mode of action of cypermethrin to fish is not clearly known. Hyperexcitability is a common reaction of fish exposed to cypermethrin (Borges *et al*., 2007) indicating that the pesticide may act through central nervous system. On the other hand, pyrethroids being lipophilic in nature, are attracted by the non-water components of gills and are thus quickly absorbed in the gill of fish (Smith and Straton, 1986) thereby causing a probability of respiratory distress. The objectives of this study were to evaluate how lethal and sublethal doses of cypermethrin acted on blood of the air breathing fish *Heteropnesutes fossilis* and how the ill effects, if any, could be ameliorated.

MATERIALS AND METHODS

Test fish: Adult specimens of *H. fossilis* (mean length 15.20 ± 0.5 cm and mean weight 20.10 ± 0.4 g) were procured from Ganga Matsya Utpadan Kendra, Kalyani, W.B. and were stocked in the laboratory in 50 L glass aquaria with 10 L of water (dissolved oxygen 6.5±0.2 mg / L, free carbon dioxide 2.1 ± 0.2 mg / L, pH 7.23±0.04, hardness 240 ± 10 mg / L, alkalinity 72 ± 3 mg / L as $CaCO_3$). The fish were acclimatized in this condition for 4 days. The acclimatized fish were then stocked in 15 L glass aquaria each with 3 L of water and 4 to 5 specimens of fish. These aquaria were assorted into three groups as per randomized block design. A diet was prepared by mixing rice bran, wheat flour, mustard oil cake, fishmeal and

vitamin-mineral mix in ratio 18.43:18.43:18.43:40.69:4.0. The mixture was ground and made into a paste by water. The paste was passed through a pelletizer with 0.2 mm diameter to make pelleted diets. The prepared diet contained 30 % crude protein (CP). Three types of prepared diet were made based on dietary supplement of ascorbic acid(AA). One group of aquaria received the prepared diet, which did not contain any ascorbic acid supplement. The second group received the same diet supplemented with 50 mg AA per 100 g diet and the third group received the diet supplemented with 100 mg AA per 100 g diet. The fish were hand fed twice daily at 9 and 16 hours up to satiation. Daily 20% of water in each vat was exchanged with fresh water. The fish were reared in this condition for two months before transferring to experimental aquaria.

Table 1. Level of dietary supplement and concentration of ascorbic acid used in the experiment

Ascorbic acid supplement (mg/100g)	Cypermerthrin (µg/L)
0	0.0
50	0.3
100	0.5

Bioassays: Bioassays were carried out in 15 L glass aquaria each containing 3 L of water and two acclimatized specimens of *H. fossilis*. Two different sets of bioassays were carried out.

Bioassay 1: 24 h bioassay with 1.0 µg / L cypermethrin in duplicate set of aquaria to determine histopathological effects of a lethal dose of cypermethrin on erythrocytes of *H. fossilis*. The basis of selecting 1.0 µg / L cypermethrin as the lethal dose is our previous research, which recorded that 24h LC_{50} of cypermethrin to *H. fossilis* was 0.96µg / L with 95% confidence limit ranging between 0.9 to 1.0 µg / L (Saha and Kaviraj, 2003). After 24h exposure the fishes were sampled and washed in clean water. The blood samples were taken directly from the heart of the sampled fishes with micro-syringe previously rinsed with heparin. A thin blood film was drawn on clean grease free slide, fixed in methanol and was stained in Leishman's stain (Bradbury, 1969).

Bioassay 2: 72 h bioassays were carried out in 18 glass aquaria (15 L) arranged in randomized block design so that there were nine treatments each with duplicate set of aquaria (Table 1). Each aquarium contained 3L of water and two fish. No food was given during the experiment. One fish was

sampled from each aquarium before the treatment of cypermethrin (0 h) and one after 72 h of treatment. Samples of blood were collected by the method described above and was directly used to determine Hb %, packed cell volume (PCV) and total erythrocyte count (TEC). Hb % and PCV values were determined respectively by cyanomethemoglobin and Wintrobe method respectively (Dacie and Lewis, 1968). TEC was determined by Neubauer's improved double haemocytometer (Fein–optic blankernburg, GDR) using Hayem's solution as RBC diluting fluid. Mean cell volume (MCV), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration(MCHC) were determined from the values of PCV,TEC and Hb. Results obtained for each parameter were subjected to one way ANOVA followed by least significance different (LSD) test (Gomez and Gomez, 1984).

RESULTS

The lethal dose of cypermethrin (1.0µg /L) caused severe damage to erythrocytes of *H. fossilis*. While blood of the control fish showed normal eleptical erythrocyte with prominent nucleus and cytoplasm, the treated fish exhibited complete dissolution of cytoplasm exposing the nucleus (Fig. 1). Haematological parameters of *H. fossilis* before exposure to cypermethrin (0 h) have been presented in Table 2, while these parameters after 72 h exposure to sublethal doses (0.3 and 0.5 µg / L) cypermethrin have been presented in Table 3. Results of one way ANOVA followed by least significant difference (LSD) test between treatments showed that there were significant differences of all haematological parameters between controls, cypermethrin treatments and between control and cypermethrin treatments. Total erythrocyte count (TEC), packed cell volume (PCV), mean cell (corpuscular) volume (MCV), haemoglobin (Hb), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC) significantly reduced (LSD; $P<0.05$) after 72 h exposure to both treatments of cypermethrin as compared to control (without AA supplement). Dietary AA (both low and high level) in control fish significantly increased all these parameters as compared to control without AA suplement. Effects of cypermethrin on haematological parameters were counteracted by high dietary AA level (100 mg / 100g diet) and all these haematological parameters increased significantly after 72 h of exposure to cypermethrin, when the treatments were accompanied by high dietary AA level, and the values became comparable (LSD: $P>0.05$) to respective control. Low dietary AA level (50 mg / 100 g diet) failed to counteract the effects of cypermethrin.

Table 2. Haematological parameters of *Heteropneustes fossilis* in different experimental groups before treatment of cypermethrin(Values are mean of three replicates ± SD)

	Control			Treatments					
mgAA[1]/100g diet	0	50	100	0	0	50	50	100	100
Cypermethrin (µg/L)	0	0	0	0.3	0.5	0.3	0.5	0.3	0.5
Parameters									
TEC [2] (10^6 / mm^3)	1.21 ± 0.01	1.24 ± 0.01	1.19 ± 0.01	1.23 ± 0.01	1.22 ± 0.01	1.23 ± 0.31	1.24 ± 0.01	1.21 ± 0.21	1.22 ±0.01
PCV [3] (%)	25.20 ± 0.01	24.13 ± 0.01	24.23 ± 0.01	24.41 ±0.01	24.52 ± 0.31	24.08 ± 0.01	24.14 ±0.31	24.25 ± 0.21	24.24 ±0.01
Hb[4] (g/dL)	9.25 ± 0.01	8.20 ± 0.01	9.10 ±0.01	9.00 ±0.01	9.21 ± 0.31	9.21 ± 0.21	9.31 ± 0.01	9.11 ±0.01	9.21 ±0.01
MCV[5] (fL)	207.23 ± 0.01	194.59 ± 0.01	203.69 ± 0.41	198.44 ± 0.01	200.98 ± 0.31	196.83 ± 0.31	195.37 ± 0.61	200.00 ± 0.01	198.77 ±0.01
MCH[6] (pg)	76.02 ± 0.01	66.11 ± 0.61	76.47 ± 0.01	73.16 ± 0.21	75.49 ± 0.01	74.87 ± 0.01	75.08 ±0.21	75.29 ± 0.51	76.11 ±0.01
MCHC[7] (g/dL)	36.68 ± 0.01	34.04 ± 0.01	37.54 ± 0.01	36.88 ± 0.01	34.66 ± 0.32	38.03 ± 0.01	38.54 ± 0.01	37.55 ±0.01	37.93 ±0.01

[1]AA = Ascorbic acid, [2]Total erythrocyte count (TEC), [3]Packed cell volume (PCV), [4]Haemoglobin (Hb), [5]Mean cell (corpuscular) volume (MCV), [6]Mean cell haemoglobin (MCH), [7]Mean cell haemoglobin concentration (MCHC)

DISCUSSION

Results of the present study revealed that plasma membrane of erythrocytes of *H. fossilis* was completely disintegrated when the fish was exposed to 1.0 µg / L cypermethrin for 24 h. Integrity of plasma membrane of erythrocyte depends on its structure and capacity to regulate transport of xenobiotics. Most fish exhibit semi mosaic model of erythrocytes membrane structure like human (Tian *et al.*, 2014). Cypermethrin, being lipophilic in nature binds easily to the lipid layer of the membrane and alters membrane integrity. Membrane transport enzymes like Na(+)-K(+)-ATPase play crucial role in the process (Thomasa and Egeea, 1998;

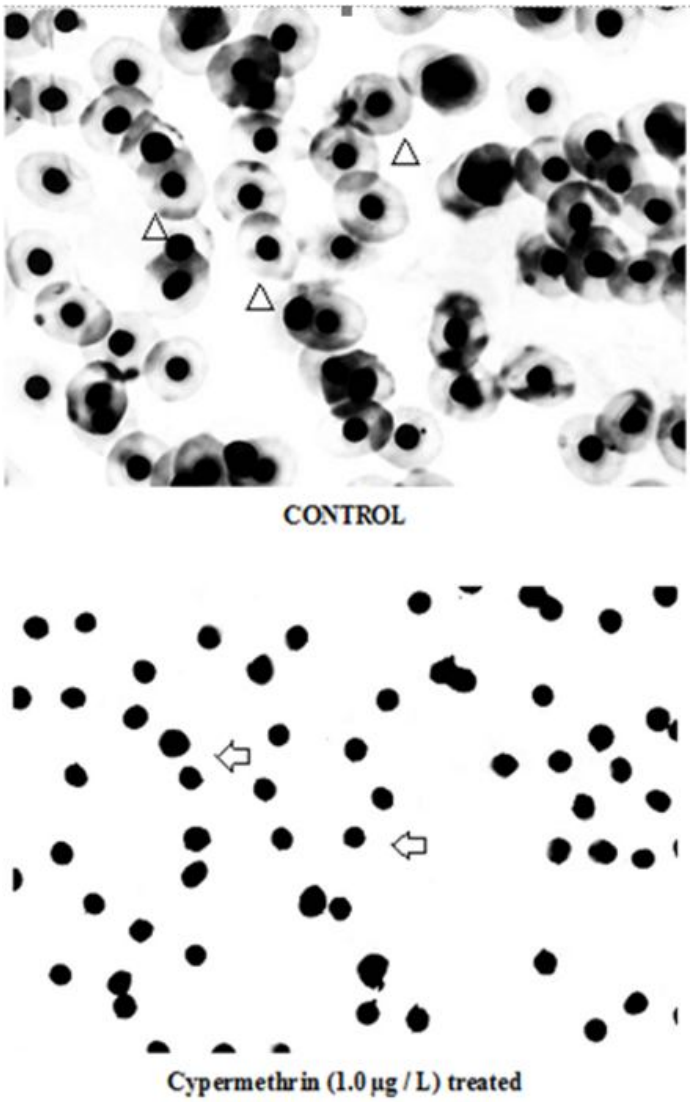

Figure1. Photomicrograph showing normal erythrocytes (arrow head) in the blood of control fish and complete dissociation of cytoplasm and plasma membrane (arrow) of the cypermethrin (1.0 µg/L) treated fish (H & E 400).

Prashnath and David, 2010 and Suvetha *et al.*, 2010). Na^+/K^+-ATPase activity in erythrocytes of the freshwater fish *Oreochromis niloticus* significantly decreased after chronic exposures to Cu, though acute exposure produced no clear effect (Canli *et al.*, 2016). There is no such report on effects of cypermethrin on erythrocytes of fish.

The haematological changes observed in *H. fossilis* after exposure to cypermethrin were most prominent. Levels of haemoglobin, red blood cells, MCH and MCHC decreased with increasing cypermethrin concentrations in those fish which did not have any protection of ascorbic acid. Decrease in Hb % may be due to increase in the rate at which Hb is destroyed or

Table 3. Haematological parameters of *Heteropneustes fossilis* exposed to control and different treatments of cypermethrin for 72 h (Values are mean ± SD)

	Control			Treatments					
mg AA[1]/100g diet	0	50	100	0	0	50	50	100	100
Cypermethrin (µg/L)	0	0	0	0.3	0.5	0.3	0.5	0.3	0.5
Parameters									
TEC [2] (10^6 / mm^3)	1.25 ±0.01	1.29 ±0.23	1.40 ±0.31	1.18 ±0.41	1.16 ±0.01	1.26 ±0.41	1.28 ±0.01	1.41 ±0.01	1.42 ±0.01
PCV [3] (%)	25.13 ±0.02	26.50 ±0.01	31.11 ±0.01	23.16 ±0.31	22.43 ±0.01	23.70 ±0.52	25.00 ±0.02	32.12 ±0.21	33.21 ±0.01
Hb[4] (g/dL)	7.81 ±0.01	8.21 ±0.01	10.07 ±0.01	3.09 ±0.01	2.05 ±0.22	6.52 ±0.31	6.80 ±0.01	11.04 ±0.21	11.80 ±0.10
MCV[5] (fL)	202.65 ±0.01	205.42 ±0.31	222.14 ±0.12	196.27 ±0.01	193.36 ±0.21	190.47 ±0.01	195.31 ±0.01	227.78 ±0.01	235.52 ±0.01
MCH[6] (pg)	63.48 ±0.01	63.63 ±0.21	71.95 ±0.01	26.18 ±0.01	17.67 ±0.01	51.74 ±0.01	53.12 ±0.51	78.36 ±0.02	83.09 ±0.61
MCHC[7] (g/dL)	31.07 ±0.01	30.98 ±0.25	32.36 ±0.31	13.35 ±0.01	9.13 ±0.01	27.16 ±0.01	27.20 ±0.26	34.39 ±0.01	35.53 ±0.21

[1]AA = Ascorbic acid, [2]Total erythrocyte count (TEC), [3]Packed cell volume (PCV), [4]Haemoglobin (Hb), [5]Mean cell (corpuscular) volume (MCV), [6]Mean cell haemoglobin (MCH), [7]Mean cell haemoglobin concentration (MCHC)

decrease in the rate of Hb synthesis (Reddy and Basamohidden, 1989). Decreased MCH is also a sign of anaemia (Shakoori *et al*., 1996). Similar decrease in Hb content as well as a decrease in total erythrocytes have been reported for cypermethrin treatment in carp *Cyprinus carpio* (Dorucu and Girin, 2001), in *Labeo rohita* (Das and Mukherjee, 2003), in rainbow trout *Oncorhynchus mykiss* (Nuri and Girgin, 2003) and air breathing teleost *Channa punctatus* (Saxena and Seth, 2002) and for deltamethrin treatment in *Cyprinus carpio* (Svoboda *et al.*, 2003). Dietary AA counteracted adverse effects of cypermethrin on haematological parameters of *H. fossilis*. AA is a nonenzymatic antioxidant and is involved in self defense mechanism of fish. However, most fish cannot synthesize AA de novo. An exogenous supply of AA diet increases in tissue reserve of AA, which in turn help to counter the stress of cypermethrin, on *H. fossilis* (Saha and Kaviraj, 2012).

It is concluded from this study that blood serves as biomarker of cypermethrin toxicity to fish. Breakdown of cytoplasmic membrane in erythrocyte is a possible mechanism of cypermethrin toxicity to fish. Dietary supplement of high ascorbic acid (100 mg / 100 g) is a useful antidote to protect fish from ill effects of sub-lethal dose of cypermethrin.

ACKNOWLEDGEMENT

The authors are thankful to Nabadwip Vidyasgar College Authority for providing laboratory support.

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Article received on10.08.2017 and accepted for publication on 26.09.2017