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Evaluation of haematological response of grass carp (C*tenopharyngodon idella)* after exposure to endosulfan

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Abstract

Pesticides used in agriculture ultimately reach the aquatic environment, get biomagnified and cause severe environmental and health problems. Present investigations have been made to assess the detrimental effects of endosulfan on the blood of exotic freshwater fish, *Ctenopharyngodon idella* (Cuv. and Val.) after exposure of the fish to two sublethal concentrations of an organochlorine pesticide, endosulfan (0.00075mg/L and 0.001mg/L) for 15, 30 and 45 days. The haematological parameters and calculated erythrocytic indices have been negatively correlated with endosulfan concentration and the experiment length. RBC count, haemoglobin content and haematocrit % values have shown a declining trend. The calculated indices like mean corpuscular haemoglobin (MCH) value and mean corpuscular haemoglobin concentration (MCHC) showed a declining trend while mean corpuscular volume (MCV) showed an increasing trend on all exposure occasions. The variation in erythrocytic indices is a good indicator of polluted aquatic environment.

Keywords: Erythrocyte count, erythrocytic indices, *Ctenopharyngodon***,** endosulfan

1. Introduction

In the recent years, human interventions in the form of industrial development, agrarian advancements and modern living styles have resulted in major changes in the aquatic ecosystems due to the release of abnormally high amounts of toxic substances like natural chemicals, mining wastes and thousands of synthetic chemicals.

The toxicants though exerted pressure on every natural resource but most sensitive and evident crack has been on water ecology because water bodies are the ultimate recipients for multitude of suspected pollutants due to direct overspray, atmospheric transport, agricultural and residential runoff, individual misuse or improper disposal. They pose significant toxicological risks to resident organisms. Their hazardous effects are more pronounced because they undergo biomagnifications; and fishes being at the top of aquatic food chain have maximum concentration and hence are the worst victims. Pesticides, without doubt have been/are playing a significant role in the enhanced cereal production to feed billions of mouths. However, they reach water bodies causing toxicity to freshwater aquatic flora and fauna and badly affecting non-target species like fishes (Rohr and Crumrine, 2005; Rohr *et al.,* 2008; Tang *et al.,* 2012) [1-3]. In fishes, the main route of entry of any pesticide/ chemical is through the gills, from where it is transported to various parts of the body via blood. The blood cell profile of fish has been considered as an important indicator of diseases and other toxicants (Tiano *et al.,* 2003) [4]. *Ctenopharyngodon idella* (Cuv. and Val.) is an exotic freshwater fish of family Cyprinidae brought from Hong Kong in December, 1959 (Jhingran, 1991) [5]. Currently it flourishes in various parts of the country successfully. Popularity of this fish stems from their ability to be cultured easily; exclusive herbivorous habit hence effective biological control on wide variety of aquatic vegetation without causing chemical pollution; being delicious and source of high quality protein. Alterations in serum proteins, serum cholesterol and serum glucose of grass carp have been observed on exposure to endosufan (Bala, 2014) [6]. Erythrocytes were found altered on exposure to Fenvalerate (Jindal and Batoye, 2015) [7]. Keeping in view the above facts, *Ctenopharyngodon idella* (Cuv. and Val.) has been subjected to toxicological studies upon exposure to two different sublethal concentrations of endosulfan for 15, 30 and 45 days to study haematological parameters. Endosulfan (6,7,8,9,10,10 hexachloro-1-5,5a,6,9,9a hexahydro,6,9, methano-2,4,3- benzodioxathiepine 3-oxide) an organochloride pesticide has no doubt been banned by Supreme Court of India but it is still being used in Punjab, India for controlling pests of cotton.

2. Materials and Methods

Live fingerlings of *Ctenopharyngodon idella* (Cuv and Val.) (12.56 ± 1.52 cm and 25.40 ± 2.53 gm) were procured from local fish farm at Barnala (Punjab, India) where farmers have put this fish to their ponds to control unwanted vegetation. After giving a dip in 0.1% $KMnO_4$ to disinfect them, they were acclimatized to laboratory conditions in the aquarium provided with dechlorinated tap water, aerators and filters for ten days. The fish were fed with grass, banana leaves etc. Endosulfan 35 EC (commercial name), manufactured by Excel industries limited, India purchased from local market, Barnala, India was used for the present investigation.

The fish were starved for 24 hours before the start of the experiment. The test fish was exposed to wide range of endosulfan concentrations prepared in dechlorinated water taken in Syntex, white coloured plastic (non-poisonous) tanks of 25 liters water capacity fitted with filters and aerators to determine LC_{50} value of endosulfan. Ten fishes were exposed to each concentration of pesticide and two replicates were used. Appropriate controls in the endosulfan free water were maintained simultaneously. These short term exposures to endosulfan gave 96 h LC_{50} values, to be 0.005mg/L.

For chronic toxicity tests, the fish were divided into three groups (10 fish each) and kept in separate tanks fitted with filters and aerators. Group I and II were exposed to two sublethal concentrations of endosulfan i.e., 0.00075mg/L and 0.001mg/L for 15, 30 and 45 days while group III was maintained as control in dechlorinated water. In order to ensure the reproducibility of the results, all tests were performed in three replicates. Water containing the toxicant was changed and aliquots of stock solution were added to each experimental tank every alternate day to maintain the required concentration of the pesticide. On completion of the stipulated exposure periods, free flowing blood from caudal vein was collected for haematological studies. The erythrocytes of fish blood were counted using haemocytometer with the improved Neubauer's counting chamber. Haemoglobin content was estimated by Sahli's haemometer. Haematocrit % was determined using wintrobe test tube, centrifuged at 3000 rpm for 30 minutes. The calculated erythrocytic indices viz. mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC) were calculated according to formulae:

$$\text{MCH (pg)} = \frac{\text{Haemoglobin (gm/dl)} \times 10}{\text{Erythrocytes } (\times 10^6 /\text{mm}^3)}$$

$$\text{MCHC (\%)} = \frac{\text{Haemoglobin (gm/dl)} \times 10}{\text{Haematocrit (\%)}}$$

$$\text{MCV } (\text{m}^3) = \frac{\text{Erythrocytes } (\times 10^6 /\text{mm}^3)}{\text{Haematocrit (\%)} \times 10}$$

Statistical analysis

All the data were expressed as mean ± S.D. Mean value for each group of fish was tested for significance by student's t-test to establish the validity of the findings.

3. Results

The haematological parameters and calculated erythrocytic indices have been negatively correlated with endosulfan concentration and the experiment length (Table 1).

RBC count has shown a regular significant decrease (p<0.05) after 15 days and highly significant decrease (p<0.01) after exposure periods of 30 days and 45 days. It has reduced from an initial 2.41 million/ml to 1.43 million/ml at the end of 45th day of exposure to endosulfan. It has been observed to decline by 11.62% (p<0.05), 20.33% (p<0.01) w.r.t. control on 15th day; by 21.34 % and 33.47 % with respect to control after 30 days and by 32.91 % and 39.66 % with respect to control on 45th day of exposure to 0.00075 mg/L and 0.001 mg/L of endosulfan respectively.

Table 1: Endosulfan induced variations in certain haematological parameters of *Ctenopharyngodon idella* (Cuv. and Val.).

Parameters	Exposure Period (days)	Control (Mean ± S. D.)	Treated (Mean ± S. D.)	
			0.00075 mg/L	0.001mg/L
Erythrocytes	15	2.41±0.128	2.13±0.482*	1.92±0.488*
(10^6/mm^3)	30	2.39± 0.414	1.88±0.845**	1.57±0.964**
	45	2.37±0.169	1.59±0.576**	1.43±0.522**
Haemoglobin	15	8.038±0.262	6.31±0.0168*	6.088±0.345*
(gm/dl)	30	7.899±0.589	6.29±0.149*	5.923±3.00*
	45	7.973±0.493	4.89±0.182**	4.36±5.854**
Haematocrit %	15	21.31±0.0865	20.68±0.47	19.83±0.313*
(Hct %)	30	21.20±0.475	19.79±0.264*	19.67±0.332*
	45	21.13±0.357	19.53±0.304**	18.86±1.034**
MCH (pg)	15	33.06± 2.74	25.57±3.92**	27.15±5.02**
	30	32.87±2.574	27.80±1.533**	29.99±1.771
	45	32.94±3.856	30.44±2.548	30.59±2.084
MCHC (%)	15	37.72 ±6.727	25.49±5.529**	24.54±5.427**
	30	37.63±6.68	26.06±5.590*	23.52±5.420**
	45	37.79±6.599	25.22± 5.501*	23.19±5.274**
MCV (m^3)	15	88.71±5.389	92.17±9.636	109.79±7.685*
	30	88.57±3.908	106.81±7.011*	122.28±6.956**
	45	88.66±7.383	124.53±6.748**	132.20±11.749**

Level of significance *p<0.05; **p<0.01. Non-significant (p > 0.05).

Haemoglobin content of fishes exposed to 0.00075 mg/L and 0.001 mg/L showed a reduction of 21.96% and 24.82% with respect to control value after 15 days; by 20.52% and 26.71% after 30 days and 30.44% and 37.84% after 45 days with respect to control value. Significant decrease (p<0.05) has been observed after 15 days and 30 days while highly significant decrease (p<0.01) after 45 days on exposure to both the concentrations.

The haematocrit reading is valuable in determining the effect of stressors on the health of fish.

The haematocrit % of the test fish has been observed to decline significantly (p<0.05) with respect to control value by 4.98% and 8.45% on 15th day and by 6.46% and 9.15% on 30th day. It showed highly significant (p<0.01) decrease of 26.08% and 31.91% with respect to control on 45th day of treatment at 0.00075 mg/L and 0.001 mg/L of endosulfan respectively.

Mean corpuscular haemoglobin (MCH) value which expresses the average Hb content in picogram (pg) of a single RBC has decreased highly significantly (p<0.01) after 15 days of exposure to 0.00075 mg/L and 0.001 mg/L (by 24.20% and 19.20%) as compared to control and also after 30 days exposure to 0.00075 mg/L (by 15.42%); but non significantly (p<0.05) after other exposures showing that % decrease become less and less with increase in duration.

Mean corpuscular haemoglobin concentration (MCHC) value refers to the percentage haemoglobin in 1dl of packed RBC. It has decreased highly significantly (p<0.01) in fishes exposed to 0.00075 mg/L and 0.001 mg/L of endosulfan (by 32.42% and 34.94%) after 15 days and only to 0.001 mg/L after 30 days (by 32.24%) as well as 45 days (by 31.37%). Significant (p<0.05) decline has been observed on exposure to 0.00075mg/L at the end of 30th and 45th days when compared to control value (Table 1). So, the decrease has been more significant in the higher concentration of toxicant.

Mean corpuscular volume (MCV) which represents *the average volume of a single cell expressed in* μm^3 has shown an increasing trend in *Ctenopharyngodon idella* (Cuv. and Val.) exposed to endosulfan. It has increased highly significantly (p<0.01) in both the concentrations after 45 days and only in 0.001mg/L after 30 days. Significant (p<0.05) increase has been observed in fishes exposed to 0.00075mg/L at the end of 30th and also exposed to 0.001 mg/L after 15 days.

4. Discussion

Erythrocytes are produced in the haemapoietic tissue, which is situated in the spleen and head kidney (Munkittrick and Leatherland, 1983) [8]. So, damage to haemopoietic tissue like kidney hence it's malfunctioning seems to be main cause of erythropenia, reduction in haemoglobin as well as haematocrit % in the present investigation. Apart from this reason various other reasons like that of structural changes in erythrocytes, inhibition of erythropoiesis, reduction in haemosynthesis due to damage to intestinal mucosa with resultant impaired absorption of iron; osmoregulatory dysfunction or an increase in the rate of erythrolysis or defect in iron metabolism in liver may also be responsible (Ellis,1976; Anandkumar *et al.,* 2001) [9-10].

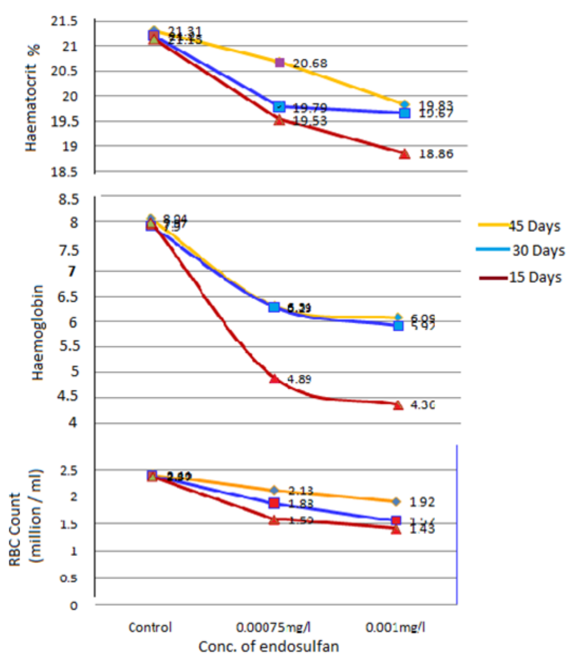

Fig 1: Variations in the RBC count, haemoglobin content and haematocrit % in *Ctenopharyngodon idella* (Val.) upon exposure to different sublethal doses of endosulfan for 15 d, 30d and 45 d.

The present results are in conformity with those of many other earlier workers who have reported erythropenia in different piscine species on chronic exposures to different pesticides. A decline in the TEC and haemoglobin % has been reported in *Ophiocephalus punctatus* exposed to endosulfan (Jenkins *et al*., 2003) [11]; in *Cyprinus carpio* exposed to concentrations of endosulfan (Chandrasekar and Jayabalan,1993) [12] ; in *Channa gachua* due to mercury poisoning (Patil and Jabde, 1998) [13]; in *Clarias batrachus* exposed to lindane and malathion (Agarwal and Tyagi, 1998) [14]; in African catfish - *Clarias gariepinus* after ochratoxin exposure (Joshi *et al.,* 2002) [15]; in *Clarias gariepinus* due to nitrite toxicity (Mousa and Khattab, 2003) [16]. Malfunctioning of kidney especially damage to haemopoietic tissue may be responsible for this effect (Bhatia *et al*., 2004) [17]. Alterations in haematological profile of grass carp have been reported even after 96h acute endosulfan toxicity (Hasan *et al.*, 2014) [18].

The perturbations in these haematological indices (increase MCV, decrease of MCH and MCHC) in the present study may be attributed to a defence against the toxic effect of endosulfan through the stimulation of erythropioesis or may be related to the decrease in RBCs, haemoglobin content and haematocrit % due to exaggerated disturbances that occurred in both metabolic and haemopoietic activities of fish exposed to sublethal concentrations of pollutants or may be due to histopathological changes that occur in erythrocytes following exposure to toxicants (Ajani *et al.,* 2007; Choudhary *et al.,* 2003; Kashiwada *et al*.,2002) [19-21]. Their alteration confirms anaemia.

The fluctuations in the MCH values clearly indicate that the concentration of haemoglobin in the red blood cells were much lower in the exposed fish than in the control over the exposure period, thus indicating an anaemic condition.

The significant decrease in the MCHC values in the exposed fish in the present investigations has been probably an indication of swelling of the red blood cells or a decrease in haemoglobin synthesis. The MCHC is a good indicator of red blood cell swelling (Wepener *et al*., 1992) [22]. The increase in MCV coupled with low haemoglobin content in present investigation indicates that the red blood cells have undergone swelling.

5. Conclusions

Values/ haematological parameters and calculated indices clearly indicate that disturbances in metabolic and haemopoietic activities of fish even at their sub lethal concentrations; and/ or due to histopathological changes in erythrocytes and haemopoietic tissue like kidney following exposure to toxicants are responsible for these alterations. Their alterations confirm anaemia and affect the metabolism of the fish further leading to death of fish. The alterations in these parameters depend on the duration of exposure as well as concentrations of chemicals. The MCHC is a good indicator of red blood cell swelling and increase in MCV coupled with low haemoglobin content in present investigation confirms that the red blood cells have undergone swelling.

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