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Impact of an Endocrine Disrupter, Carbaryl on the Fresh Water Fish Catla catla

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Abstract

Freshwater fish, *Catla catla* were subjected to endocrine disrupting compound (EDC), a carbamate pesticide (carbaryl) for an acute concentration. The median lethal concentration (LC_{50}) was 14.99 mg l^{-1} for 24 h. Haematological parameters like red blood cell (RBC), white blood cell (WBC) haemoglobin (Hb), hematocrit (HCT), mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) was significantly altered in the carbaryl treated fish. The current study points that the pesticide carbaryl was toxic to freshwater fish and the results from the haematological parameters serve as the useful nonspecific biomarkers.

Keywords: Carbaryl, *C. catla*, Blood, Haematological Parameters

1. Introduction

The aquatic ecosystem is often get polluted by anthropogenic pollutants, pesticides which are anxious compounds, widely used in agricultural activities (Ramesh *et al.*, 1994). These are reported to cause severe neurotoxic damage to aquatic systems (Beauvais *et al.*, 2001). According to (NRC, 1992) changes in the behaviour that may spoil subsequent existence of revealed animals may be due to neurotoxic injury. Neurotoxicity assessment of a contaminant needs the evaluation of exposure and characterization of dose-response. An interaction between the various agents in a biological system was measured using the biomarkers.

The carbamate (CB) group of insecticides are being used for several decades and be used in all over the world (Ferrari *et al.*, 2007). Carbaryl is a carbamate pesticide (CB) and forms an active ingredient of many commercial insecticides (USEPA, 2003). Fish is an important form of highly nutrient food and also a useful biological indicator of pollution of aquatic system (Agrahari *et al.*, 2006). The haematological parameters otherwise reflects as biomarker of acute concentrations (Pimpao *et al.*, 2007).

The primary objective is to study the effects of carbaryl as a major endocrine disruptor of the Indian major carp. The present study is thus intended to analyze hematological parameters and changes such as Hb, HCT, RBC and WBC count, can be induced by accidental factors such as imprison and sampling as well as long term factors such as exposure to disease and environmental contaminants (Drastichova *et al.*, 2004; Carvarlho and Fernandez, 2006). Symptoms of anemia and fluid volume disturbance are used for the estimation of hemoglobin.

According to (Koundinya and Ramamurthi, 1979; Varo *et al*., 2003; Kuster and Altenburger, 2007), there are different studies of haematological alterations in the effect of organochlorine and organophosphorous pesticides on fish and the reports available on the effect of carbaryl, an carbamate pesticide on different clinical, biochemical, haematological studies are scant. In total fresh water fish production of India *L. rohita*, *C. catla*, and *C. mrigala* are the dominant species of Indian major carps and the current investigation is decided to study the effect of an endocrine disrupter, carbaryl on haematological features of *C. catla*

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2. Material and Methods

2.1 Fish

C. catla (Hamilton) fingerlings ranging 6.5 ± 0.4 g weights and 6.5 ± 2 cm body length were procured from Tamil Nadu Aliyar Fish Farm, India. It was brought and adapted to the lab for 18 days. At the time of acclimatization, they were fed with groundnut oil and rice bran oil cake.

2.2 Water

Daily water was changed and before 24 h of commencement the experiment, feeding was stopped. In present study chlorine free tap water was used which comprises the physico-chemical features following APHA, 1998; temperature (27.2 ± 1°C), pH (7.2 ± 0.08), dissolved oxygen (6.2 ± 0.04 mg l^{-1}), total hardness (17.4 ± 0.4 mg l^{-1}), salinity (0.4 ± 0.02 ppt). Following the method of APHA 1971 a static bioassay was made dividing the fish randomly into 2 groups with continuous aeration.

2.3 Toxicant

Carbaryl (Sevin) an insecticide was marketed by Bayer Crop Science Ltd, Mumbai, India, containing 50% W.D.P. 1gm of carbaryl was dissolved in 1000 ml of water for the preparation of Stock.

2.4 Determination of 24 Hours Lethal Concentration 50 of Toxicity of Carbaryl

According to finney, 1978 probit analysis method Lethal Concentration 50 of *C. catla* for 24 hours was calculated. Five different concentrations of carbaryl like 12.5, 13.5, 14.5, 15.5 and 16.5 mg l^{-1} were used for finding LC_{50} value for 24 hours. 10 fishes were introduced into the glass tanks by selecting randomly from the stock. In toxicant free water a control with 3 replicates was maintained. Fish survival/mortality were recorded after 24 hours and immediately the dead fish was removed. Bioassay experiment was carried out by the prior withheld of feeding. 50% mortality of fish after 24 hours in 14.99 mg l^{-1} concentration was taken as the lethal concentration (LC_{50}).

2.5 Acute Toxicity Test

Acute experiment was conducted exposing 30 fish, using 50 L aerated glass aquarium containing the corresponding amount of carbaryl (three replicates per treatment). A separate control was also performed in triplicate using glass aquarium without carbaryl. After 24 h exposure, fish were sacrificed moreover heart was punctured and blood samples were obtained. By centrifugation at 9,000 rpm for 22 minutes of the blood, plasma was obtained. Haematological parameters comprised red blood cell (RBC), white blood cell (WBC) haemoglobin (Hb), hematocrit (HCT), mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) were observed.

2.6 Hematological Assessments

RBC and WBC were determined using hemocytometer following the method of Rusia and Sood, 1992; hematocrit was determined by microhematocrit following the method of Nelson and Morris, 1989. Following Drabkin, (1946) Cyanmethaemoglobin method, hemoglobin content of the blood was estimated. Erythrocyte indices such as mean cell volume (MCV), mean cell hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC) were calculated from RBC, Ht, and Hb using the formulas.

2.7 Statistical Analysis

By following the student's t test differences between groups at each sample were assessed. The standard errors (SE) data were expressed as means. The level of significance ($p<0.05$) was considered.

3. Results

3.1 Physico-Chemical Parameters

Physical and chemical features have effect on the toxicity of pesticides in animals according to Lloyd (1965) and Tabata (1969). In the present study, all the physical and chemical parameters of the water are maintained below the threshold level.

The physico-chemical characteristics of water used for experiments were dissolved oxygen 6.8 ± 0.002 mg/l, PH 7.4 ± 1 and temperature 27.0 ± 1.0 °C.

3.2 LC_{50} Concentration

In water pollution behavioural toxicity serves as the tool for the hazard assessment. Behavioural responses like erratic movement, abundant mucus secretion, wide opening of the operculum and loss of equilibrium were noticed in the fish exposed to 24 hours LC 50 concentration. Fish were apparently fighting hard for breathing and swam slantingly. Then jerking movement was observed before they died.

Table 1. Acute concentration in the hematological profiles of *Catla catla* treated with carbaryl

Parameters	Control Value	Experiment Value	Percent Change	Calculated 't' Value
Hemoglobin (g/dl)	3.284 ± 0.007	2.264 ± 0.015	-31.05	0.576
Hematocrit (%)	9.110 ± 0.006	6.092 ± 0.004	-33.12	6.423*
Erythrocytes (1,00000/cu.mm)	0.458 ± 0.012	0.208 ± 0.014	-54.58	0.754
Mean Cellular Volume (cubic micra)	198.90 ± 0.605	292.88 ± 0.036	+47.24	292.408*
Mean Cellular Haemoglobin (picograms)	71.71 ± 0.051	108.88 ± 0.040	+40.67	108.362*
Mean Cellular Haemoglobin Concentration (g/dl)	36.05 ± 0.178	37.18 ± 0.026	+3.13	37.149*

3.3 Acute Hematological Findings

The hemoglobin and hematocrit level of fish exposed to carbaryl for 24 h (Table: 1) are significantly lower when compared to control groups. The exposure to carbaryl elicited hyperactivity characterized by agitated movements, rapid pectoral and opercula movement, excess mucous secretion and erratic swimming.

4. Discussion

Carbamate insecticides, especially carbaryl are extensively utilized in forestry to control piercing and sucking insect pests (Zinkl *et al* 1991). The chemical has short environmental persistence and rapid insecticidal action. In the present study *Catla catla* was exposed to acute (14.99 mg/l) carbaryl toxicity for 24 hours.

Catla catla exposed to 14.99 mg/l of carbaryl in the present study showed hyper chronic and macrocytic anaemia due to the elevated levels of haemoglobin, haematocrit and erythrocytes. When the fish was exposed to pesticides it causes anaemic condition which was reported by several workers (Mandal *et al* 1985, Schwaiger *et al* 1997). Changes in the composition of blood can be used as biomarkers of dysfunctional physiological conditions. In this study, the main changes in red blood cells included a decreasing Hb. However, mean values recorded in P. lineatus for these variables are within the normal range reported for this species.

According to (Mandal and Lahira 1985; Sen *et al* 1992) animals exhibit stress in response to leucocytic condition the changes in leucocytosis with heterophilia and lymphopenia were noted. Unspecialised resistant response, activated by physiological pressure and a reduced state of health were recorded due to the elevation of white blood cell density in the current study.

However, in the current investigation the increase of MCH and MCV was observed in carp and it may be due to symptoms of hypo chronic microcytic anaemia, accompanying iron deficiency in the body. During acute treatment increase in MCHC value may be due to hereditary spherocytosis. Due to substantial utilization and hazards of carbamates, the neurotoxicity of environmental contaminants assessment is vital and it is concluded in the present study.

The blood parameters alterations are important in diagnosing the structural and functional status of fish exposed to toxicants. Since the pesticide carbaryl is classified as one of the Endocrine Disrupter Chemical (EDC) and the usage of this pesticide may be avoided.

5. Conclusion

From the current study, it is summarised that in aquatic ecosystems, carbaryl contamination is dangerous and it has a serious impact on the hematological features of *C. catla*.

When this insecticide is used in controlling of piercing and sucking insects the fact should be taken into consideration. Moreover in the field of environmental biomonitoring the taken-up parameters are effectually used as potential biomarkers to the freshwater fish toxicity of carbaryl.

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