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Full Length Research Paper

Effect of atrazine (Herbicide) on blood parameters of common carp *CYPRINUS CARPIO* (Actinopterygii: Cypriniformes)

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In the present study an attempt was made to investigate the acute toxicity of atrazine (ATR) a herbicide on an economically important fish, *CYPRINUS CARPIO.* In which, 24 h median lethal concentration (24 h LC_{50}) of herbicide, atrazine to *C. CARPIO* was 18.5 ppm. Due to fish exposure to 24 h LC_{50} of atrazine for 24 h, red blood cells (RBCs) count (-63.17%), hemoglobin (-27.35%), plasma glucose (-6.78%) and plasma protein (-18.73%) levels were decreased; whereas white blood cells (WBCs) count (+3.73%) was enhanced. The differences in haematological and biochemical values were statistically significant ($p < 0.05$). However, WBC count was not significantly changed. The alterations of the above parameters could be used as an important tool for the assessment of pathological conditions of fish.

Key words: Atrazine, acute toxicity, hematology, biochemical, *Cyprinus carpio*.

INTRODUCTION

Due to rapid industrialization, application of synthetic fertilizers and use of various insecticides and pesticides, the natural water resources are fast degrading in the water quality. Aquatic ecosystems that run through agricultural or industrial areas have high probability of being contaminated by run off and ground water leaching by a variety of chemicals (Todd and Leuwen, 2002). Agricultural pesticides are released into the atmosphere by the spray drift, post application, volatilization and wind erosion of soil (Qiu et al., 2004). Ventura et al. (2008) reported that pesticides presents in aquatic environments can affect aquatic organisms in different ways. In India, more than 70% of the chemical formulations are employed in agricultural practices and to find their way to freshwater bodies, ultimately affect non-target organisms (Bhatnagar et al., 1992).

The use of herbicides to control aquatic weeds has applied in fish management where they are used in aquatic habitats especially rice fields and some fish farms (Wu et al., 1980). ATR has been one of the most widely used herbicides to control broad- leaf weeds in corn or crops, including green vegetables (Cui et al., 2002). After spraying on crops, it can enter watercourses, because of its high mobility through soil (Waring and Moore, 2004). Hussein et al. (1996) pointed out that ATR reaches aqua-tic environments due to proximities of the agricultural country sides to the water places, or directly due to the careless application in such environments. After reaching the environment the atrazine or triazine based herbicides are not degraded by microbial or hydrolytic process (Gamble et al., 1983). However, WHO (1996) reports pointed out that atrazine can be degraded in surface water by photolysis and microorganisms and the half-lives of 20 - 50 days at 20 - 25°C have been found under laboratory conditions and increasing at lower tempera-tures (USEPA, 1988).

It has been detected in natural (Solomon et al., 1996; Power et al., 1999) and surface waters at concentrations exceeding 0.1 gl^{-1} in some areas (Environment Agency, 1997) and also accumulated in a variety of tissues (Du Preez and van Vuren, 1992). Many authors have reported the impact of atrazine on the physiology and metabolism of aquatic organisms particularly on fishes (Prasad et al.,

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1991; Hussein et al., 1996) and metabo-lism (Grobler et al., 1989; Srinivas et al., 1991; Prasad et al., 1995; Phyua et al., 2006).

Fish are one of the most widely distributed organisms in an aquatic environment and being susceptible to environmental contamination may reflect the extent of the biological effects of environmental pollution in waters. Monitoring of blood parameters, both cellular and non-cellular may have considerable diagnostic value in assessing early warning signs of pesticide poisoning (Pant et al., 1987). In India, ATR still is one of the most widely used herbicides controlling broad leave weeds and grasses. The possible effects of ATR on aquatic eco-system have stimulated studies to understand the mechanisms and measurements of the toxic effects of it to aquatic organisms. Hence, the present study is designed to study the acute effect of ATR as a herbicide on blood parameters of a freshwater teleost fish *C. carpio*.

MATERIALS AND METHODS

C. carpio was selected for the present investigation and the healthy specimens were collected from Tamil Nadu Fisheries Development Corporation Limited, Aliyar Fish Farm, Tamil Nadu, India. Fish were acclimatized to laboratory conditions for about 15 days before the commencement of the experiment. During this period, fish were fed *ad libitum* with rice bran and oil cake in the form of dough daily. Water was replaced every 24 h after feeding in order to maintain a healthy environment with enough oxygen. The aquarium water was analyzed for physico-chemical characteristics according to APHA (1998) (Table 1).

Five hundred fish were stocked in a large cement tank (4 m × 6 m × 3 m) after cleaning and disinfected with potassium permanganate. Fish with an average weight of 6 g and length about 7 - 8 cm were selected for the experiment. The LC_{50} value of the herbicide atrazine (ATR), (2-chloro-4-ethylamino-6-isopropylamino-1,2,3-triazine) (18.5 ppm) to fish was calculated following the method of Finney (1978) with a confidence limit of 95%. The acute toxicity experiment was carried out in two circular glass tanks, filled with 40 l of water. A normal pH (6.3) and ATR concentration of 18.5 ppm (LC_{50} 24 h) were maintained throughout the experiment. Twenty fish, which were already withheld from feeding for 48 h, were introduced into each tub. The control was maintained in two circular glass tanks with 20 fish per tub. After 24 h, 40 randomly sampled fish, 20 each for the control and ATR treated groups were used for the haemato-biochemical assay. Care was taken to minimize disturbances to the animals. After the stipulated time period (24 h) fish from control and ATR treated tanks were sacrificed and blood was collected by cardiac puncture using heparinised syringes and kept at low temperature (4°C). All analyses were performed on pooled blood samples. Whole blood was used for the estimation of red blood cells (RBCs), white blood cells (WBCs) and haemoglobin (Hb) content. RBCs and WBCs were counted by the method of Rusia and Sood (1992). Haemoglobin was estimated by cyanmethemoglobin method (Drabkin, 1946). Then the pooled blood samples were centrifuged for 15 min at 10 000 rpm, the plasma was withdrawn and transfer-red into clean vials for plasma glucose and protein estimation. Glucose was estimated by *O*-Toluidine method of Cooper and Mc Danial (1970). Protein was estimated according to the method of Lowry et al. (1951). For the experiment and control group (toxicant free water), three replicates were maintained. The data were ana-

Table 1. Showing the physico-chemical parameters of the tab water.

Physico-chemical parameters	Values
Temperature	$28^{O}C \pm 1.0^{O}C$
pH	6.3 ± 0.2 units
Dissolved oxygen	6.4 ± 0.01 mg/l
Salinity	0.5 ± 0.02 ppt
Total hardness	19.00 ± 0.05 mg/l

lysed statistically at $P < 0.05$. To test their significance the *t*-values were calculated by Student's *t*-test.

RESULTS AND DISCUSSION

The calculated LC_{50} for 24 h with a confidence limit of 95% of atrazine (ATR) to the carp, *C. carpio* was 18.5 ppm indicating the moderate toxicity of ATR to the fish.

During above exposure period the fish shows various behavioral responses like increased opercular movement, mucous secretion, jerky movement, floating on the sides, hypersensitivity showing violent erratic and fast swimm-ing etc. The abnormal behaviour of the fish indicates the toxic effect of ATR on central nerves system (CNS) and cardiovascular system as suggested by Antychowicz et al. (1979). Documented effects of ATR in fish include a slow down in reflexes, swimming activity and feeding (Hussein et al., 1996). Puigdoller et al. (2007) reported that fish Atlantic salmon exposed to 100 gl^{-1} ATR had reduced food consumption after 10 and 15 days of exposure. Hussein et al. (1996) suggested that these behavioral changes were the result of decreased acetyl cholinesterase activity.

The effects of environmental stressors on the peri-pheral blood of fishes are well documented in the literature. ATR is toxic; often bioaccumulative and persistent (Fernando et al., 1992). The blood alterations of carp *C. carpio* exposed to acute concentration of ATR is shown in Figure 1. During acute treatment RBC count (-63.17%), hemoglobin (-27.35%), plasma glucose (-6.78%) and plasma protein (-18.73%) levels were lowered when compared to that of their control group whereas WBC count (+3.73%) was increased. The differences in haematological and biochemical values were statistically significant ($p < 0.05$). However, WBC count was not significantly changed. Hussein et al. (1996) reported decreased RBCs number, hemoglobin concen-tration and haematocrit percentage of *Oreochromis nitoticus* and *Chrysichthyes auratus* when exposed to 3 and 6 mg/l ATR. Puigdoller et al. (2007) reported significant increase in hematocrit in Atlantic salmon when exposed to ATR. Prasad et al. (1991) found that damage of the gill lamellae causes decreased respiratory capacity in *Tilapia mosambica* exposed to 1.1 mgl^{-1} ATR. Horn

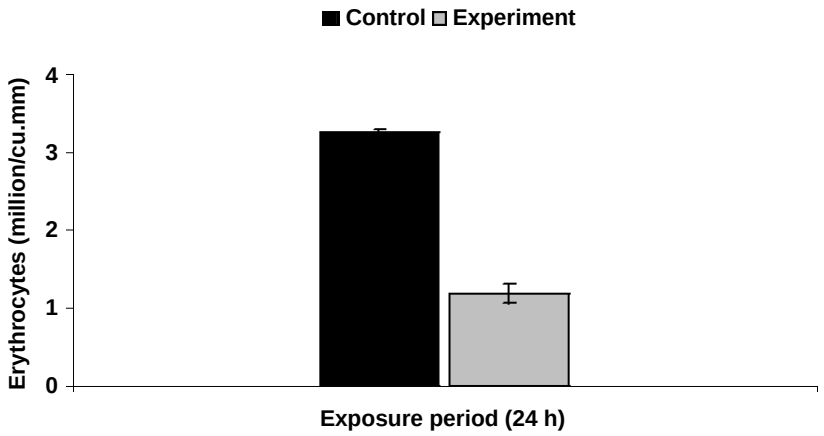

Figure 1a. Bar Diagram showing changes in the erythrocytes levels of Common Carp *C. carpio* during acute concentration of Atrazine (ATR) (Herbicide). Values are means ± S.E. of five individual observations, ($p < 0.05$).

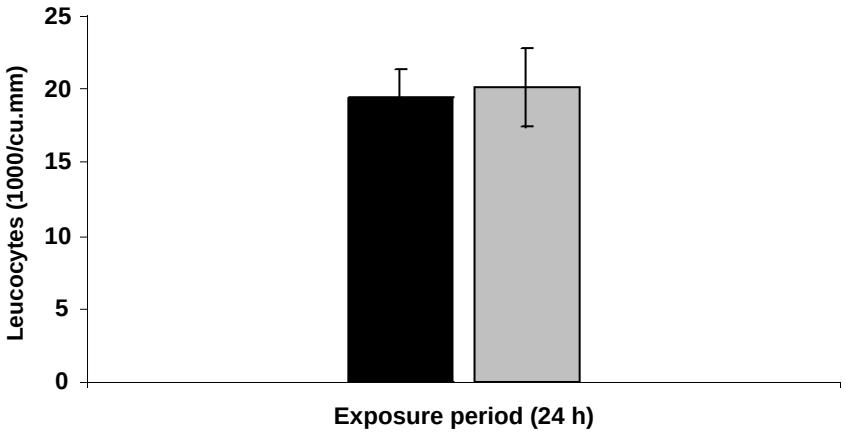

Figure 1b. Bar Diagram showing changes in the leucocytes levels of Common Carp *C. carpio* during acute concentration of Atrazine (ATR) (Herbicide). Values are means ± S.E. of five individual observations, ($p < 0.05$).

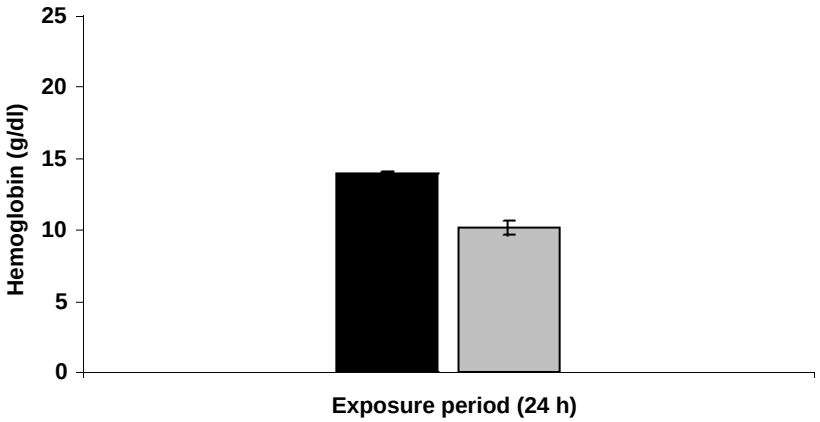

Figure 1c. Bar Diagram showing changes in the hemoglobin levels of Common Carp *C. carpio* during acute concentration of Atrazine (ATR) (Herbicide). Values are means ± S.E. of five individual observations, ($p < 0.05$).

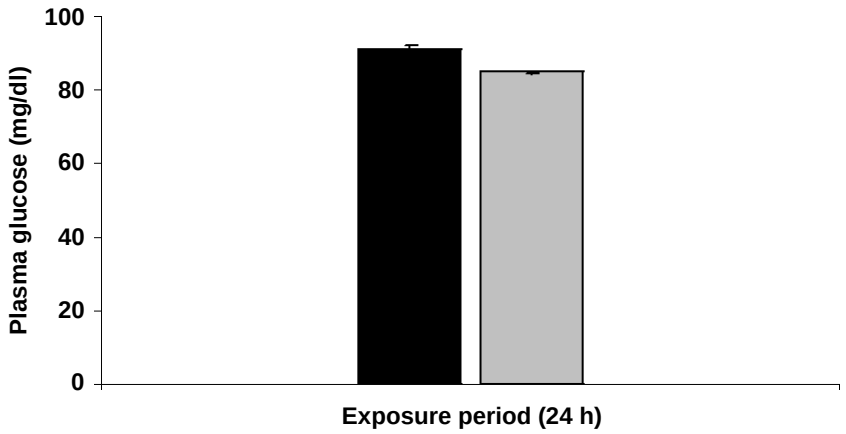

Figure 1d. Bar Diagram showing changes in the plasma glucose levels of Common Carp *C. carpio* during acute concentration of Atrazine (ATR) (Herbicide). Values are means ± S.E. of five individual observations, ($p < 0.05$).

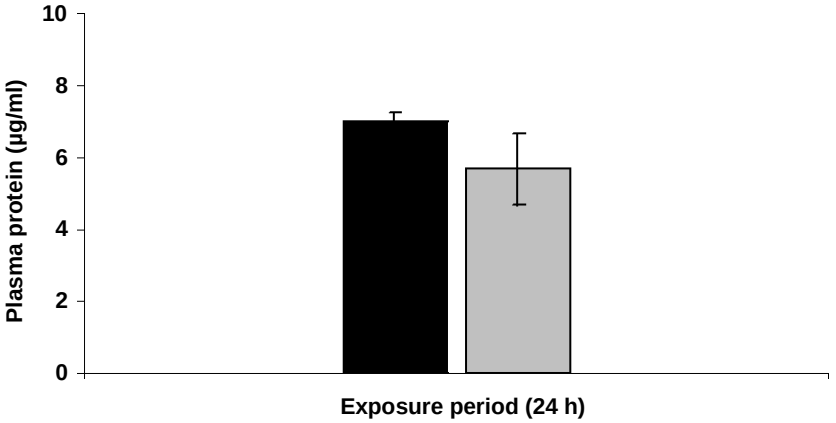

Figure 1e. Bar Diagram showing changes in the plasma protein levels of Common Carp *C. carpio* during acute concentration of Atrazine (ATR) (Herbicide). Values are means ± S.E. of five individual observations, ($p < 0.05$).

Hanke (1980) found a decline in the number of erythrocytes after exposure of *C. carpio* to 0.1 mg/l atrazine. Ventura et al. (2008) showed a high frequency of micronuclei and nuclear abnormalities in *O. niloticus* exposed to different concentrations of ATR.

Erythrocyte level was found to be depressed in fishes subjected to stressful conditions. Changes in the erythrocyte profile suggest a compensation of oxygen deficit in the body due to gill damage and the nature of the changes shows a release of erythrocytes from the blood depots (Drastichova et al., 2004). Inhibition of erythropoiesis and increase in the rate of erythrocyte destruction in hematopoietic organs is the cause of decrease in RBC count (Joshi et al., 2002). Rehwoldt (1978) found significant decrease of RBCs, Hb and packed cell volume (PCV) in ATR exposed fish species and indicated the toxic effect of ATR on spleen, liver and anterior kidney. In the present study, the significant decrease in RBCs and hemoglobin content might have resulted from the lowering of the oxygen content of the water due to the presence of atrazine in the test media. Further reduction in the total erythrocyte count (TEC) might have attributed to a decrease in the erythropoietic activity of the kidney or to the haemodilution resulting from impaired osmoregulation across the gill epithelium. Leucocytes are involved in the regulation of immunological function and their numbers increase as protective response in fish to stress. Such an increase in total leucocyte count (TLC) occurs by the increase in lymphoperisis and/or enhanced release of lymphocytes from lymphoid tissues (Johansson-Sjobeck and Larsson, 1978). Fink and Salibian (2005) reported that WBC increase could be due

to an induced proliferation as a result of the chemical toxicity, of pluripotential hematopoietic cells that in turn may be a consequence of a depletion circulating differrentiated cells. The increase in WBC count in the present study indicates the stress condition of the fish caused by ATR which might have produced hypoxia and gill damage.

Changes in carbohydrate metabolism measured as plasma glucose can be used as general stress indicators in fish. Reduction in serum glucose levels after exposure to toxicants appears to be caused by hypoxic conditions leading to an excess utilization of stored carbohydrates. Hussein et al. (1996) found significant decrease in serum glucose. This decrease could be attributed to the toxic effect of ATR on the liver (Braunbeck et al., 1992). In the present study, the decreased level of plasma glucose during acute treatment might have resulted from hypoxic condition caused by the herbicide ATR. Further, Hussein et al. (1996) indicate that reduction in food intake of ATR treated fish could be also considered another possible reason for decrease in plasma glucose.

The concentration of protein in the serum of fish has been used as an indicator of their general state of health. Das et al. (2004) reported that the higher energy demand might have triggered an increase in protein catabolism, a process in which both blood and structural protein are converted to energy, thereby reducing serum protein. They further reported that dilution of plasma volume after haemolysis and shrinkage of RBC could also cause a small reduction in protein percentage in serum. Failure of haemopoiesis is a characteristic indicator of kidney damage. Kidney damage causing increased renal excretion of blood protein may also have contributed to the depletion of serum protein in the fingerlings. However, Hussein et al. (1996) reported that the decrease of total protein in ATR treated fish *Oreochromis niloticus* and *Chrysichthyes auratus* was mainly due to globulin, explaining the toxic effects of ATR on the immune system of these fishes. In the present study, the reduction of plasma protein of fish from acute treatment indicates the toxic effect of ATR on spleen, liver and kidney. Gluth and Hanke (1985) found that carp exposed to 100 gl^{-1} ATR for 72 h had significantly lower plasma protein concentrations, which suggested a haemodilution effect operating in these fish.

From the present study, it is concluded that, atrazine (ATR) has a profound influence on the blood profiles of the treated fish. Hence, the use of the herbicide ATR should be minimized and these parameters could be effectively used as potential biomarkers of herbicide toxicity to *C. carpio*.

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