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RESEARCH ARTICLE

BIOCHEMICAL CHANGES IN THE BLOOD OF INDIAN MAJOR CARP *Catla catla* (HAMILTON) EXPOSED TO SUBLETHEL CONCENTRATION OF MONOCROTOPHOS

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ABSTRACT

Indian major carp *Catla catla* were exposed to sublethal concentration of monocrotophos to determine the role of pesticide on fish physiology. Fish were exposed to sublethal concentration (0.0250mg/l) for acute (5 days) and subchronic (30 days). Blood biochemical parameters such as Total protein, Total glucose, Urea nitrogen, Creatinine, Cholestrol, Triglycerides, Bilirubin, AST and ALT levels were estimated in the control and experimental fishes. Significant changes in the biochemical parameters were obtained in the experimental group compared with the control. The total protein and total glucose decreased in the acute and sub chronic exposures compared with control; where as a significant increase in urea nitrogen, creatinine, bilirubin, cholesterol, triglycerides, AST and ALT levels were noticed. The changes in the biochemical profiles shows monocrotophos induce alterations in the blood, which may be used as an index for the toxic role of pesticide on the aquatic organisms.

INTRODUCTION

Pesticides have become an increasingly serious source of chemical pollution of the environment due to their extensive usage in agriculture. In natural aquatic environments, alterations in the chemical composition, such as those caused by pesticide contamination, can affect the freshwater fauna, particularly fish. Fish are particularly sensitive to environmental contamination of water and therefore, pollutants may significantly damage certain physiological and biochemical processes when they enter the organs of fishes (Agrahari *et al*., 2007). Indeed, fish have been extensively used as bioindicators for environmental pollutants in evaluations of the water quality of aquatic systems (Yonar and Sakin, 2011). The exposure of fish to several types of chemical agents may induce changes in several hematological parameters, which are frequently used to evaluate fish health. Haematology has been widely used for the detection of physiopathological alterations following different stress conditions. (Yonar *et al*., 2012). Therefore the present report is aimed to study the biochemical changes in the blood of *Catla catla* exposed to sublethal concentration of monocrotophos one of the extensively used organophosphorus (OP) insecticides in agriculture.

MATERIALS AND METHODS

Catla catla were collected from local commercial fish ponds and acclimatized to laboratory conditions by keeping them in cement tanks. The fish were fed with supplemented commercial feed. For the experimental fishes weighing about 20-25 g were selected. Feeding was stopped one day before commencing of experiments in order to minimize the quantity of excretory products in the test tank. The physico chemical conditions of well water used in the present study has the following characteristics; Dissolved oxygen 7.5 - 8 ppm; Salinity 0.3 - 0.5 ppm; Alkalinity 252 mg/l as $CaCO_3$; Hardness 373mg/l; as $CaCO_3$ PH: 7.4 to 7.7 ;Temperature : 28 ± 2°C. Based on the percent mortality values the median lethal concentration values of monocrotophos for 6, 12, 24, 48, 72, 96 and 120 hours for *Catla catla* were calculated by following the method of (Litchfield and Wilcoxon, 1949). The experiments were carried out in sublethal concentration (0.0250mg/l) of monocrotophos for 5 days (acute) and 30 days (sub chronic).

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Collection of blood sample

Blood was collected from control and monocrotophos treated groups by cardiac puncture. Plastic disposable syringe fitted with 26 gauge needle which was already moistured with heparin was used. The collected blood was expelled into separate heparinised plastic vials and kept immediately on ice. The blood was centrifuged for 20 min at 2000 rpm and plasma was separated for the biochemical estimation. Total protein contents were analyzed by (Lowry *et al*., 1951) Aspartate aminotransferase (AST) Alanine aminotransferase (ALT) was assayed by (Wotton, 1964). Glucose was estimated by the method of (Trinder, 1969) using reagent Kit. Cholesterol was assayed by (Pande and Tambi, 1963). Bilirubin was assayed by (Doumas *et al*., 1983). The biochemical indices were determined with commercially available reagent kits including creatinine, blood urea nitrogen, and triglycerides based on colorimetric reaction in automatic analyzer according to the instruction of the manufacturers.

Statistical analysis

The data was statistically analyzed by statistical package SPSS version 16, in which data was subjected to one way ANOVA. Comparisons were made at the 5% probability level.

RESULTS AND DISCUSSION

Fishes exposed to sublethal concentration of monocrotophos for acute (5 days) and subchronic (30 days) exposures shows significant changes in the blood biochemical profiles (Fig. 1-9). The protein level found to be 3.17 ± 0.2, 2.92 ± 0.09, and 2.28 ± 0.08 for control, 5 and 30 days respectively. The total protein level significantly ($P<0.05$)

decreased in 30 days and in 5 days to some extent when compared with control. In the present study consistent reports also made in other fishes. *Catla catla* exposed to cypermethrin decreased the protein, albumin,globulin and albumin-globulin ratio (Vani *et al*., 2012). In *Cyprinus carpio* exposed to atrazine shows decrease in the plasma protein level (Ramesh *et al*., 2009). Das and Mukherjee, (2003), reported the serum protein level decrease in *Labeo rohita* treated with cypermethrin. In *onchorynchus mykiss* exposed to diazinon shows significant decrease in plasma protein (Banaee *et al*., 2011). Contradictory to above, increase in protein level also reported., *Saccobranchus fossilis* exposed to chlordane increase the protein level in the blood (Verma *et al*., 1979), *Cirrhinus mrigala* exposed to neem extract increase the plasma protein (Saravanan *et al*., 2011). Thus the decline in serum total protein, albumin and globulin may also be due to a high degree of haemodilution under the stress of pollution (Mazeoud *et al*., 1977). The glucose level found to be 63.5 ± 0.7, 54.5 ± 0.8 and 50.0 ± 0.52 for control, 5 and 30 days. The glucose decreased significantly ($P< 0.05$) compared with control. In the present study decrease in blood glucose was noticed when *Catla catla* exposed to monocrotophos for 5 and 30 days. Similar reports also reported for other fishes, *Channa punctatus* exposed to monocrotophos significantly decrease the glucose level (Agrahari *et al*., 2007), *Cyprinus carpio* to atrazine decreased glucose level (Ramesh *et al*., 2009). Increase in glucose level also reported in fishes exposed to other pesticides. *Clarias batrachus* exposed to three organophosphorus pesticides viz., carbaryl, carbamate and phorate for 24, 72, 120 and 168 hours increase the serum glucose level (Jyothi and Narayan, 1999). In *Labeo rohita* exposed to Cypermethrin increase in the blood glucose was noticed (Das and Mukherjee 2003). *Saccobranchus fossilis* exposed to chlordane increase the glucose level (Verma *et al*., 1979). In *onchorynchus mykiss* exposed to diazinon shows significant increase in plasma glucose (Banaee *et al*., 2011). Saravanan *et al*., (2011), reported that plasma glucose increase in the *Cirrhinus mrigala* exposed to neem leaf extract. Glucose is one of the most important sources of energy for the animals and glucose has been studied as an indicator of stress caused by physical factors (Manush *et al*., 2005) in particular pollutants (Svobodova *et al*., 1991). Stress increases the glucose content in blood because of intensive glycogenolysis and the synthesis of glucose from extra hepatic tissue proteins and amino acids (Almeida *et al*., 2001). Decreased plasma glucose concentrations can cause hypoglycemic condition. This was apparent in the present study following acute and subchronic exposure. Hypoglycemia in fish may also reflect stress induced response (Chowdhury *et al*., 2000). Significant decrease in blood glucose level in some freshwater fishes following the exposure of monocrotophos has been shown (Yasmeen *et al*., 1991). In the present study hypoglycemia in *Catla catla* may probably due to the rapid utilization of blood glucose during hyperexcitability, tremors and convulsions, which are characteristic behavior of organophosphate pesticide toxicity in fish (Ozer *et al*., 2008).

The blood urea nitrogen increased in the exposed group compared with control and found to be 0.698±0.01, 1.48±0.05 and 2.62± 0.11. The creatinine levels were 0.4080 ± 0.012, 0.46 ± 0.005 and 0.950± 0.011 for control, 5 and 30 days respectively. A two fold increase was noticed in 5 and 30 days compared with control. . Increase in urea nitrogen and creatinine in *Carassius auratus* (Zhang *et al*., 2007). Banaee *et al*., (2011), found no changes in creatinine in *onchorynchus mykiss* exposed to diazinon. The creatinine test has been usually used to diagnose impaired kidney function and to detect renal damage (Toffaletti and McDonnell, 2008). Gilbert *et al*., (1989), reported that plasma creatinine was usually not an accurate biomarker to distinguish dysfunction in kidney tissue. The cholesterol level increased significantly in 5 and 30 days ($P<0.05$) and found to be 122.5 ± 0.8, 145.4 ± 0.9 and 200.7 ± 0.9 for control, 5 and 30 days. Like cholesterol, triglycerides also increased significantly in the exposed fishes, and found to be 123.8 ± 1, 149.7 ± 0.8 and 203.0 ± 09 for control, 5 and 30 days. Compared with control, AST and ALT level increased significantly ($P<0.05$). The AST level found to be 63.9 ± 0.5, 68.8 ± 0.3 and 76.5 ± 0.4 and ALT 77.0 ± 0.50, 81.3 ± 0.3 and 89.6 ± 0.4 for control 5 and 30 days respectively. *Channa punctatus* exposed to monocrotophos increase the cholesterol, GOT, GPT and decrease in triglyceride levels in the blood (Agrahari *et al*., 2007). AST and ALT level increased in *Onchorhychus mykiss* exposed to dimethoate (Dogan and Can, 2011). *Labeo rohita* exposed to distillery effluent increased the AST and ALT concentration (Dhanapakiyam *et al*., 2006). In *onchorynchus mykiss* exposed to diazinon shows significant increase in plasma AST and ALT (Banaee *et al*., 2011).The transaminases are a group of enzyme catalyzing interconversion of amino acids and a-ketoacids by transfer of amino groups and elevated activity of these tissue-specific enzymes has been used to diagnose damage to liver. Monocrotophos exposure resulted in duration dependant induction of serum AST and ALT enzyme activities (Singh *et al*., 1993). Since, increase in the activities of blood transaminase has been attributed to tissue damage, particularly in the liver (Gupta and Paul, 1978, Palanivelu *et al*., 2005). It is generally accepted that an increase of these enzyme activities in the extracellular fluid or plasma is a sensitive indicator of even minor cellular damage (Van der *et al*., 2003, Palanivelu *et al*., 2005). *Saccobranchus fossilis* exposed to chlordane decrease the cholestrol level in the blood (Verma *et al*., 1979). Cholesterol is a steroid lipid found in the cell membranes of all body tissues and transported in the blood plasma. Increase or decrease in cholesterol concentration in the blood plasma indicating the hyper or hypo cholesteremia. Triglycerides are used to evaluate nutritional status, lipid metabolism, and its high concentrations may occur with nephritic syndrome or glycogen storage disease (Yang and Chen, 2003). The bilirubin level significantly elevated in the 5 and 30 days compared with control and found to be 3.0 ± 0.05, 3.56 ± 0.05, 3.78 ± 0.03 for control, 5 and 30 days. In the present study similar increase in bilirubin also reported in *Clarias batrachus* (Jyothi and Narayan, 1999). Bilirubin is primarily formed from the breakdown of red blood cells (Lehninger, 1987).

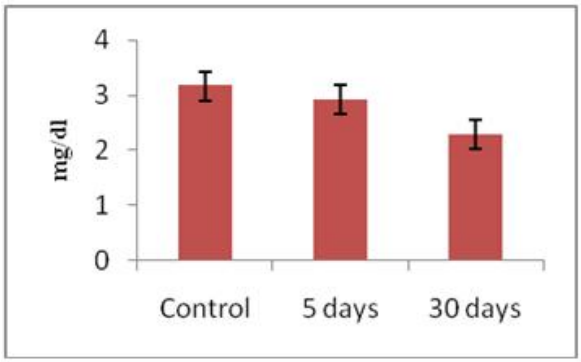

Fig.1. Changes in Blood Protein of *Catla catla* exposed to sublethal concentration of monocrotophos for 5 and 30 days

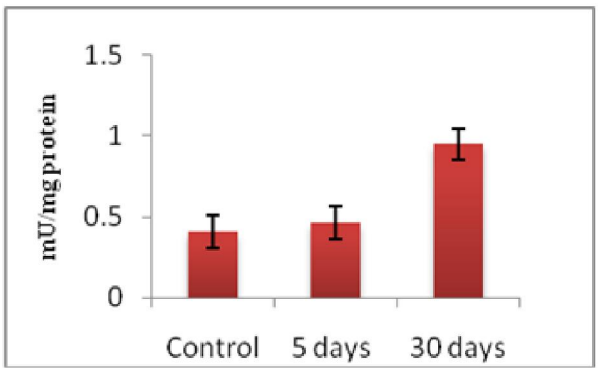

Fig.2. Changes in Blood Glucose of *Catla catla* exposed to sublethal concentration of monocrotophos for 5 and 30 days

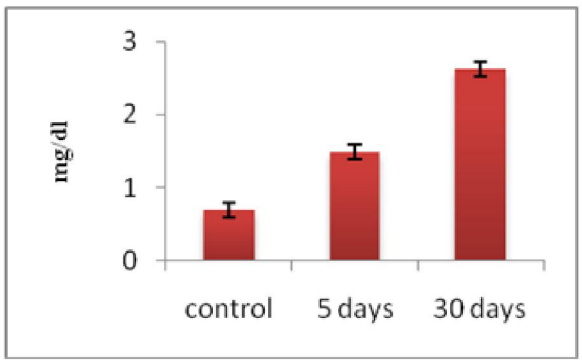

Fig.3. Changes in Blood Urea nitrogen of *Catla catla*. Exposed to sublethal concentration of monocrotophos for 5 and 30 days

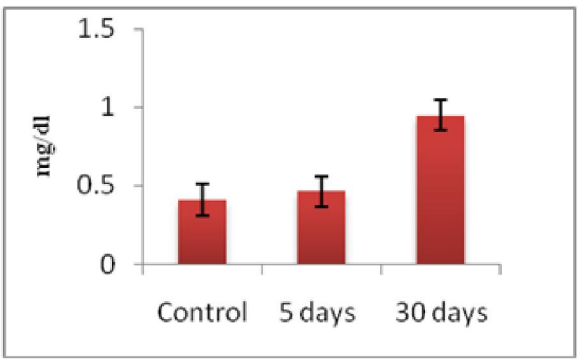

Fig.4. Changes in Creatinine of *Catla catla*. Exposed to sublethal concentration of monocrotophos for 5 and 30 days

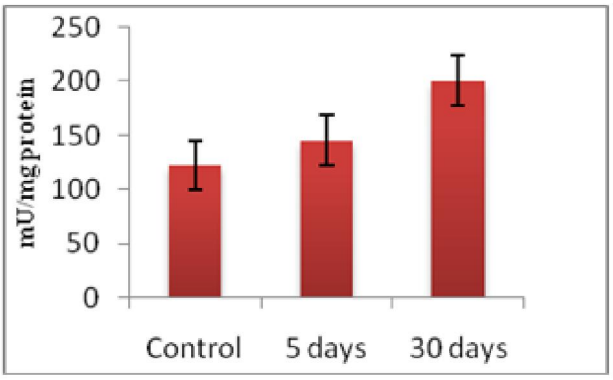

Fig.5. Changes in Blood Cholesterol of *Catla catla*. Exposed to sublethal concentration of monocrotophos for 5 and 30 days

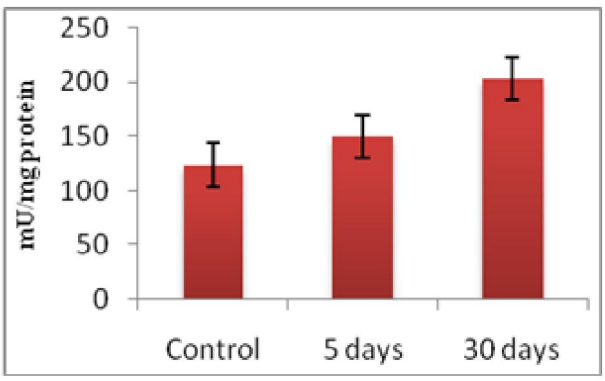

Fig.6. Changes in Blood Triglycerides of *Catla catla*. Exposed to sublethal concentration of monocrotophos for 5 and 30 days

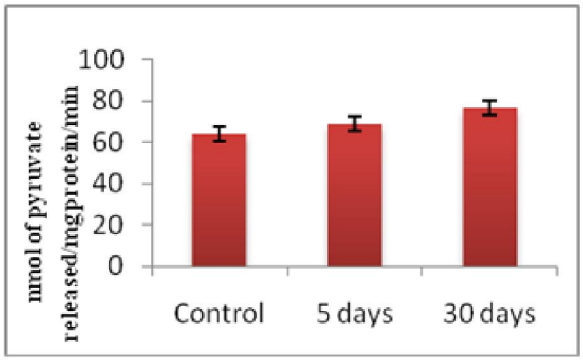

Fig.7. Changes in Blood AST of *Catla catla*. Exposed to sublethal concentration of monocrotophos for 5 and 30 days

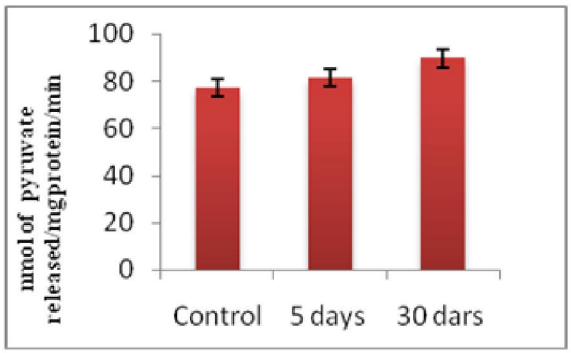

Fig.8. Changes in Blood ALT of *Catla catla*. Exposed to sublethal concentration of monocrotophos for 5 and 30 days

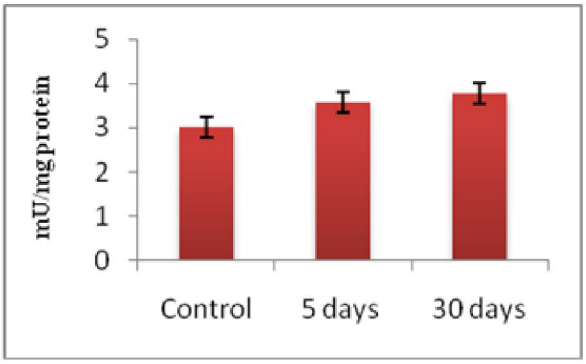

Fig.9. Changes in Blood Bilirubin of *Catla catla*. Exposed to sublethal concentration of monocrotophos for 5 and 30 days

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