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Changes in blood parameters induced by chronic exposure of carbamate pesticides on fresh water fish *Cirrhinus mrigala* (Ham.)

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Abstract : *Cirrhinus mrigala* is an Indian major carp commonly preferred in composite fish culture. The common carp *Cirrhinus mrigala* was exposed to organophosphorous pesticide fenthion to determine the LC_{50} values at different time interval and its sublethal effects on the hematological and biochemical parameters. LC_{50} values at varying times were recorded as high as 2.5 mg/l (24hr), 2.2 mg/l (48hr), 1.8mg/l (72hr) and 1.5mg/l (96hr). Fishes exposed to sub lethal concentrations (0.38, 0.19, 0.96 mg/l) for 60 days revealed that the pesticide caused alterations in blood parameters. It is found that it decreases RBC count (6-29%), hemoglobin concentration (7-28%), ESR (11-22%) and clotting time (7-32%), were as WBC count (12-31%) and platelet count (9-43%) were found to be increased. Plasma level of glucose and lactic acid was higher and protein level in blood plasma was lower in exposed fish when compared to control. In conclusion, the changes observed indicate that hematological parameters can be used as an indicator of carbamate related stress in fish on exposure to elevated level.

Key words: *Cirrhinus mrigala,* Carbamate, glucose, haematology, protein

INTRODUCTION

Carbamates are a class of insecticides structurally and mechanistically similar to organophosphate (OP) insecticides. Carbamates are N-methyl Carbamates derived from a carbamic acid and cause carbamylation of acetylcholinesterase at neuronal synapses and neuromuscular junctions.

Carbamate pesticides have been synthesized and sold commercially since the 1950s. Currently, carbamates are one of the major classes of synthetic organic pesticides and are used annually on a large scale worldwide. Carbamates are mainly used in agriculture, as insecticides, herbicides, fungicides, nematocides, acaracides, molluscicides, or sprout inhibitors. In addition, they are used as biocides for industrial and other applications, and in household products.[1] Most of the carbamates have high melting points and low vapor pressures. They are usually distributed in aqueous environments because of their high solubility in water. Studies have shown that carbamates and their degradation products are potential contaminants of the environment and food resources. There is increasing evidence indicating that carbamates may spread throughout ecosystems by leaching and runoff from soil into ground and surface water. Carbamates are identified as potential leachers because of their high water solubility. They may also enter environmental water from industrial wastes, accidental spillage and dumping. Carbamates are on the priority lists released by the US Environmental Protection Agency (EPA). Carbofuran, an anti cholinesterase

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carbamate, is commonly used. The Common trade name where carbamate is used are Aldicarb Temik, Carbaryl, Prokoz, Sevin and Larvin.

Study of hematological parameters can indicate physiological response to contaminated environment.[2] Biochemical parameters can show the impact of water pollution on fish.[3] Mrigal *C. carpio* is an economically important fresh water fish and commonly cultured in many parts of the world. In the study, an attempt was made to investigate the toxicity on blood cell count, glucose, lactic acid, protein, hemoglobin, ESR and clotting time in the blood plasma of *Cirrhinus mrigala* exposed to different sub- lethal concentrations of carbamate for 60 days exposure.

MATERIALS AND METHODS

Specimens of *Cirrhinus mrigala* were procured from fisherman of the area. The length and weight ranged from 12- 14cm and 20-25 g respectively. They were kept in aquaria for two weeks to get acclimatized to laboratory conditions. During the period of acclimations all fishes were fed with commercial fish food twice daily. After acclimation, 30 aquaria each containing 30L water were stocked with ten fish per aquarium. Different concentrations (0.01, 0.1, 0.2, 0.40, 0.6, 0.8, 1.0 mg/l) of carbamate purchased from Bayer India Ltd, were prepared by adding the required volume of acetone. A control set was run with the same number of fish and the same volume of acetone without adding carbamate. The experiments were then run in triplicate. The water in the aquaria was altered daily. The lethal concentration LC_{50} for 24, 48, 72 and 96 hr was computed by the Probit method.[4] Acute toxicity study revealed that toxicity of carbamate does not increase with time. Therefore toxicant concentration selected were ¼, 1/8 and 1/16 of 96 hr LC_{50} (0.387, 0.193 and 0.096mg/l). All the concentrations were prepared on the same day and parallel control group were maintained in similar way.

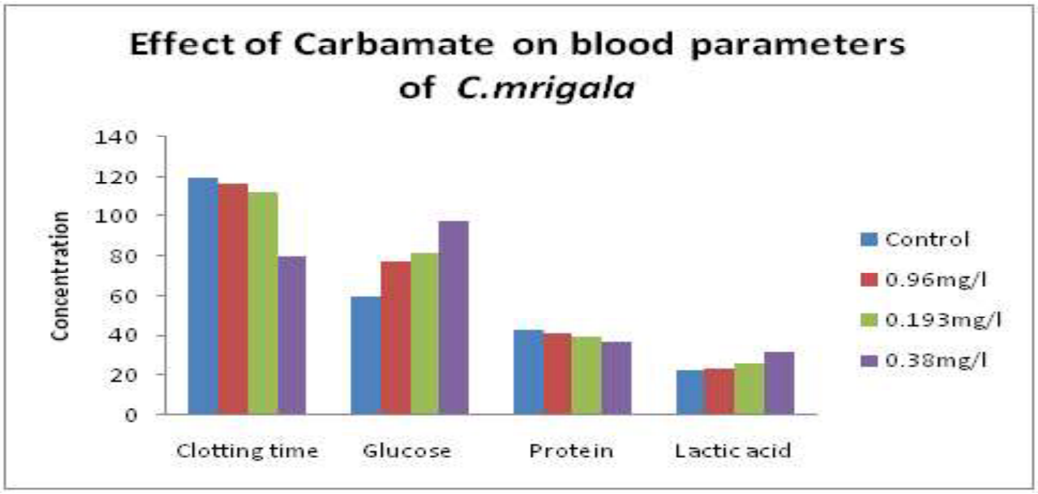
Fishes from the aquarium were removed after 60days of exposure during the experimental period. Blood samples were collected in heparinized vials by cutting the caudal peduncle .Collected fresh sample of blood was used for analysis. Hemoglobin content in the blood was estimated by acid hematic method.[5] Coagulation time was determined by Lee White method.[6] Red blood cell count was done by hematocytometric method using Neubaur's counting hemocytometer, thrombocyte and white blood cell count by indirect method suggested by Lucas and Jumroz (1961)[7] and ESR by Westergen method. The remaining blood was centrifuged at 6000 rpm for 10min at 4^oC and the collected plasma was stored at -20^oC till analyzed (Zubair Ahmad, 2011)[4]. The glucose, proteins and lactic acid content in blood plasma was assayed by Folin Wu, Lowry and Berker and Summerson method mentioned by Hawk *et al.*, (1965)[5].

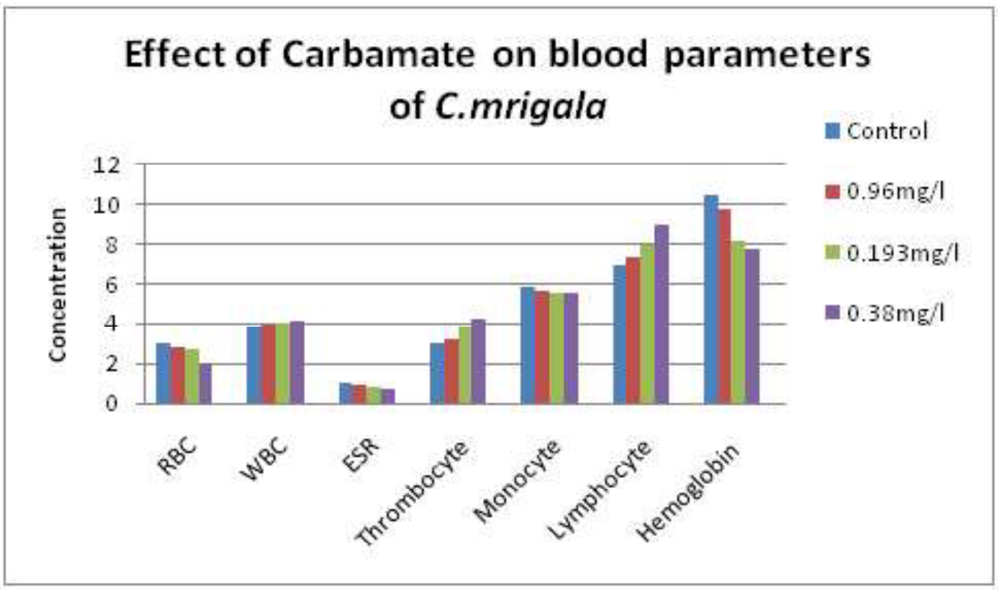
RESULTS AND DISCUSSION

LC_{50} values were calculated from the mortality rate recorded for 24, 48, 72 and 96hrs by straight line graphical interpolation method. The 95% confidence limit of LC_{50} value and slope function of the line has been calculated according to the method suggested by Litchfield and Wilcoxon (1949)[8]. The magnitude of toxic effects of pesticides also depend on length and weight, corporal surface/bodyweight ratio and breathing rate.[9,1] According to the report given by Sheela (1982)[10] carbamate is a neurotoxic agent. Oh *et al.* (1991)[11] and Murthy (1986)[1] reported that toxicity is due to detoxification and absorption. Toxicity varies with respect to species, size of fish and duration exposure (Dutta *et al.* 1995)[12].

The blood is a unique mirror in which all the internal process taking place in an organism are reflected. The observation on different hematological parameters provide a good deal in diagnosing the effects of environmental stress on an animal and in turn would give an insight in the changes induced in the circulating fluid. Investigators have also identified changes in several hematological parameters as indicators of metal exposure.[13] However, the present findings indicate that in Mrigal sub- lethal chronic exposure to carbamate altered the blood parameters.

The fish exposed to different concentration of carbamate showed increase in glucose and lactic acid and decrease in protein, hemoglobin and clotting time. Values obtained were compared with the control fish. It is documented that under stress condition, fish become hyperactive perhaps to get out of the stressful medium and would require an increased amount of oxygen to meet their energy demand, secondly fish secrete increased amount of mucus to coat the body especially gills to get relief from the irritating pollutants fig 8. This in turn reduces the gaseous exchange through the gill, thus an





increased utilization of oxygen. This condition causes glycolysis in the liver and muscles of carbamate exposed fish.

The change in the protein content in the blood plasma of Mrigal due to chronic exposure observed. The reduction in protein content was dependent on the concentration of carbamate. Among the three concentration selected the highest concentration (0.38mg/l) seem to be the most highly potent and cause significant change (-28.10%) in protein level. This could be due to the impairment of protein synthesis. Verma *et al*. (1979)[14], Singh *et al.* (1982)[9] Dutta *et al.* (2003)[15], Bucher *et al.* (1990)[3] reported similar decrease in protein content in blood of different species of fish when treated with different pollutants. Similar response was noted in other fresh water fish exposed to acute toxic level of pesticides.[16,17] The pesticide may change the function of vital organs like liver and kidney disrupting the homeostatic condition of the body. Decrease in protein content may be due to their degradation and the product which may in be fed to TCA cycle through amino transferase system to cope up with the carbamate stress. Secondly reduction in protein content level could also be attributed to histopathological damage to stomach and liver leading to its impaired functioning.

Elevation in lactic acid level was due to anaerobic pathway lead by the fish to meet the increased demand for energy under pollution stress. Similar increase in lactic acid content in *Heteropneustes fossils* was reported by Sastry and Subhadra (1982)[18]. It is noted that fish was hyperactive and this has attributed to impaired gill function. Disturbance in coagulate mechanism whether acquired or inherited may not only from alteration of coagulation but also from abnormal fibrinolysis activity of the plasma. Depression in coagulation time between 125 and 85 sec in *Saccobranchus fossils* and between 140 and 95sec in *Labeo rohita* was observed after exposure to chloradane by Verma *et al.* (1979)[14]. The decrease in clotting time can be attributed to deletion in protein content and also due to histopathological damage caused to liver (figure 6). The LC_{50} value of Mrigal was estimated as 2.5, 2.2, 1.8 and1.5 mg/l for 24, 48, 72 and 96 hrs. The LC_{50} values recorded for the fry of tilapia was 2.8 mg/l for 24 hrs by Soman (1987)[19]. The difference in values may be related to the difference in susceptibility and tolerance related to its accumulation, biotransformation and excretion. Differences in metabolic pathway among species may result in varied patterns of biotransformation, leading to more or less toxic metabolites.[20] Blood parameters generally of fish are suitable food for evaluating the effects of chemicals.[21] In the present study, sub-lethal exposure to carbamate altered blood parameters. The fish exposed to different concentration of carbamate indicated significant reduction red blood cells (RBC's), hemoglobin as compared with the untreated control group. The decrease was 6.6% (0.096mg/l), 19% (0.193mg/l), 29% (0.38mg/l) and the decrease in hemoglobin was 7 % (0.096mg/l), 19.62% (0.193mg/l) and 28% (0.38mg/l) after 60 day exposure. In contrast to this, the white blood cell (WBC's) recorded significant increase after exposure to carbamate. WBCs counted to 31.25%, 25%,and 12.25%at 0.096mg/l,0.193mg/l and 0.38mg/l respectively. Thrombocyte count level also increased to 9.3% (0.096mg/l),25% (0.193mg/l) and 43% (0.38mg/l). A complete blood count provides detailed information about three types of blood cells, red blood cell RBC's, white blood cells and platelets. Furthermore, hematological characteristics have been widely used in the diagnosis of variety of diseases and pathologies induced by pesticides and several others.[22-25]

Red blood cells are very important for transport of oxygen and hemoglobin concentration is directly correlated with RBC count. This is due to synergistic link among these blood parameters in all vertebrates. This close correlation between erythrocyte count and hemoglobin concentration was also reported for other vertebrates including man by Harris (1972)[26]. In the present study reduction in RBC count may be due to increase rate of break down RBC or reduction rate of formation or RBCs. Similar to the present state a decrease in number of RBC, hemoglobin and hematocrit value of diazinon exposed fish was reported on Banaee *et al.* (2008)[27].

WBCs formed; either migrate to key organs such as spleen, lymph nodes, gut, or enter the blood. The increased number of leucocytes can occur abnormally as a result of a toxic chemical. Sudden increase of WBC may be due to the activation of the animal's defense mechanism and the immune system. Several chemical compounds including insecticides, generate antibodies due their interference with immune system which could be the reason for increase in WBC due to the exposure to toxic pollutants. Higher WBC count could be related to the inflammation of the stomach observed (fig. 6). Mossa (2004)[24] and Mansour and Mossa (2005)[25] in rats showed that treatment with pesticides marked by elevation in the animal's defense mechanism and immune system.

CONCLUSION

From the 96hr LC_{50} recorded, it can be concluded that carbamate is moderately toxic to Mrigal. Exposure to chronic sub- lethal concentration of carbamate resulted in significant hemato- biochemical alterations. These changes suggest that the treated fish are faced with severe metabolic stress. The results clearly indicate that usage of carbamate in the field may be a threat to fishes as well as humans. The fact is that carbamate is metabolized to toxic form in fish presumably has environmental and health implication for its use as pesticide.

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