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Changes in Carbohydrate Metabolism in *Cyprinus Carpio* Var. *Communis* during Short Term Exposure to Dimethoate

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ABSTRACT

The present study deals with the sub-lethal effect of Dimethoate on the energy metabolism of the common carp, *Cyprinus carpio* var. *communis*. Analysis of various parameters such as blood glucose, liver glycogen and total liver protein was made after 24 hrs and 96 hrs of dimethoate exposure. Mortality studies showed that the sub-lethal level, Lc_{50} of *Cyprinus carpio* var. *communis* for 96 hr exposure was 1.61 mg/l for dimethoate. Mean blood glucose values elevated significantly ($p \leq 0.05$) after exposure to the sub lethal concentration of dimethoate. However, other parameters like liver glycogen and total liver proteins reduced significantly with the increased concentration and period of exposure of dimethoate. The period of exposure and concentration of dimethoate had significant affect on the changes in various carbohydrate parameters.

Key words; Common carp, Dimethoate, Toxicity, Blood glucose, liver glycogen, Carbohydrate metabolism.

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INTRODUCTION

Pesticides are of great value to agriculture and public health. However, when they are used in an unsustainable manner, they impact the environment negatively. These pesticides are carried into aquatic ecosystem by surface runoff from sites of application, where they enter the organisms through food webs and also through contact in water. Therefore the health of aquatic ecosystem is being adversely affected because they serve as ultimate sink for the pesticides. Organophosphorous pesticides degrade rapidly, but under conditions of low temperature, low moisture, high alkalinity and lack of suitable microbial degrades, it may remain biologically active in system for months or longer. Among different classes of pesticides, organophosphorous pesticides are more frequently used, because of their high insecticidal property, low mammalian toxicity, less persistence and rapid biodegradability in the environment [1]. Like other organophosphates, Rogor is also an acetylcholine esterase inhibitor [2, 3]. Therefore works primarily as nerve poison which is reflected in uncoordinated abnormal behavior of the fish soon after exposure to pesticide. The main aim of the present study was to evaluate the toxicity of various sub lethal doses of Dichlorvos in *Cyprinus carpio* var. *communis* based on the results of biochemical investigation.

MATERIALS AND METHODS

Alive, healthy and disease free specimens of *Cyprinus carpio* var. *communis* used in the present study, were collected from the local water bodies (Dal lake & River Jhelum) and acclimatized in the laboratory for 15 days in 50 litre glass tanks before they were used for experimentation. Fishes were put in four separate glass aquaria, keeping one aquarium as control. Each aquarium containing fresh dechlorinated water was fitted with artificial aerators to ensure proper aeration. During acclimatization fishes were fed with a commercial fish food once daily. Leftover food in the aquarium was removed daily when water of the aquarium was changed. Dead fish if any was removed immediately to avoid fouling of water. After two weeks of acclimatization, fishes were starved for 24 hrs prior to exposing them to different concentration of pesticide. The three sub lethal concentrations of 50%, 60% and 70% of Lc_{50} were calculated for experimental studies. Four aquaria each filled with about 40 liters of fresh dechlorinated water and 10 acclimatized fishes were taken. The four groups of fishes were treated for 96 hrs as follows.

Group 1	Control
Group 2	Exposed to 50% Lc_{50} of Dimethoate
Group 3	Exposed to 60% Lc_{50} of Dimethoate
Group 4	Exposed to 70% Lc_{50} of Dimethoate

To study the effects of Dimethoate, five fishes from each group were sacrificed by cephalic stunning, at the end of 24 hrs of and 96 hrs of exposure. The liver and muscles were collected from the sacrificed fishes. However, blood was removed directly from the heart of the fish by heparinized syringes before killing the fish. In the liver and muscles of *Cyprinus carpio* var. *communis,* glycogen and total proteins were estimated as per the standard methods of [4] and [5] and the blood glucose level was estimated as per God Pod method [6]. The average length and weight of the fishes used were 15±1.5cm and 105±6.5gms respectively.

RESULTS AND DISCUSSION

Mortality studies showed that the sub-lethal level, Lc_{50} of *Cyprinus carpio* var. *communis* for 96 hr exposure was 1.61mg/l for dimethoate. The minimum effective doses 50% of Lc_{50} (0.80mg/l), 60% of Lc_{50} (0.96mg/l) ,70% of Lc_{50}(1.13mg/l) were calculated for experimental purposes. Biochemical estimation of blood glucose, glycogen and total proteins in liver of control as well as treated *Cyprinus carpio* var. *communis* were carried out. The following results were obtained after exposing the fish to different concentrations of the pesticide:

Blood Glucose: The quantity of mean glucose in the blood of the normal fish (Control) was 58mg/100ml. At the end of 24hr exposure to 0.80mg/l, 0.96mg/l and 1.13mg/l sublethal concentration of Dimethoate, the value of plasma glucose significantly ($p<0.05$) changed to 74mg/100ml, 82mg/100ml and 90mg/100ml respectively. The quantity of plasma glucose showed significant ($p<0.05$) variation from 58mg/100ml in control fish to 76mg/100ml, 88mg/100ml and 96mg/100ml in fish exposed for 96 hr to 0.80mg/l,0.96mg/l and 1.13mg/l dimethoate respectively (Table 1).

Liver Glycogen: The change in the level of glycogen was also assessed in the fishes exposed to Dimethoate. The quantity of glycogen in the control fishes was significantly ($p<0.01$) reduced from 1.76mg/g to 1.50mg/g,1.44mg/g and 1.34mg/g after 24hr exposure to 0.80mg/l, 0.96mg/l and 1.13mg/l of Dimethoate respectively. After 96hr exposure to 0.80mg/l, 0.96mg/l and 1.13mg/l of Dimethoate, the quantity of glycogen significantly ($p<0.01$) reduced to 1.27mg/g,1.18mg/g and 1.09mg/g respectively as compared to 1.76mg/g present in control fish (Table 1).

Total Liver Proteins: The protein content of the control fish was found to be 335mg/g. After exposure for 24hrs to 0.80mg/l, 0.96mg/l and 1.13mg/l of Dimethoate, the total liver proteins significantly ($p<0.01$) reduced to 310 mg/g, 305 mg/g and 280 mg/g respectively.

Table 1. Change in certain biochemical parameters in fish *Cyprinus carpio* var. *communis* after Dimethoate exposure. Values are mean ± standard deviation of four replicates. + =(P<0.05); ++ =(P<0.001),when students 't' test was applied between control and treated groups. Values in the parenthesis are percent change with control taken as 100%.

Exposure	Plasma glucose mg/100ml			
Time(hr)	**Control**	**0.48mg/l**	**0.66mg/l**	**0.85mg/l**
24 hrs	58±3.36 (100)	$74±3.55^{+}$ (127)	$82±3.74^{+}$(141)	$90±3.88^{+}$ (155)
96hrs	$58±2.74^{+}$ (100)	$76±3.16^{+}$ (131)	$88±3.16^{+}$ (152)	$96±3.22^{+}$(165)
Exposure	**Liver glycogen (mg/g)**			
Time(hr)	**Control**	**0.48mg/l**	**0.66mg/l**	**0.85mg/l**
24 hrs	1.76±0.44 (100)	$1.50±0.32^{++}$ (85)	$1.44±0.28^{++}$ (82)	$1.34±0.23^{++}$ (76)
96hrs	1.76±0.26 (100)	$1.27±0.24^{++}$ (72)	$1.18±0.18^{++}$(67)	$1.09±0.16^{++}$ (62)
Exposure	**Total liver proteins (mg/g)**			
Time(hr)	**Control**	**0.48mg/l**	**0.66mg/l**	**0.85mg/l**
24 hrs	335±5.30(100)	$310±4.35^{++}$ (92)	$305±4.20^{++}$ (91)	$288±3.90^{++}$ (86)
96hrs	335±5.15 (100)	$295±4.50^{++}$ (88)	$278±4.05^{++}$ (83)	$258±3.60^{++}$ (77)

At the end of 96hrs of exposure to Dimethoate, the change in the total proteins of liver were studied. The value of the liver proteins calculated in control fish was 335mg/mg. After 96hrs exposure to 0.80mg/l, 0.96mg/l and 1.13mg/l of Dimethoate, the total protein concentration in liver significantly ($p<0.05$) reduced to 295mg/g, 278g/mg and 258g/mg respectively (Table 1).Thus the results of the biochemical parameters under study revealed a time and dose dependant change in biochemical parameters.

Fish mortality due to pesticide exposure mainly depends upon its sensitivity to the toxicant, its concentration and duration of exposure. The Lc_{50} value of dimethoate for certain air breathing teleosts are reported to be very high, as in *Clarias batrachus* it is 65 mg /l for 96hrs [7], whereas in *Heteropneustes fossils* very high Lc_{50} value for 24, 48, 72 and 96 hour dimethoate exposure is recorded as 3.38, 3.23, 3.08 and 2.98 mg/l [8]. In contrast, the carps are very sensitive to Dimethoate and record very low Lc_{50} values. In the present study 96hr Lc_{50} value of Dimethoate for *Cyprinus carpio* var. *communis* was found to be 1.61 mg/l. Thus in air breathing fishes Lc_{50} values are very much higher than in carps for the same pesticide, probably because the fishes with accessory respiratory organs can adaptively shift towards aerial breathing in the contaminated water.

Carbohydrate is an important biochemical constituent of an animal tissue. They not only act as building blocks of the cell but also serve as a reservoir of chemical energy to be increased or decreased according to organism need. The results obtained in the present study showed that the glycogen level significantly decreased at all sublethal concentrations of Dimethoate. These results are in accordance with the results of [9] who reported that sub lethal concentration of certain organophosphorus pesticides caused glycogenolysis which produced hyperglycemia in the African food fish, *Tilapia mossambica* and the Indian cat fish, *Heteropneustes fossils*. The possible depletion of glycogen may be due to its rapid utilization to meet the energy demands and to enhance the physiological processes for metabolizing and eliminating the toxicants. Since dimethoate is the inhibitor of acetylcholinesterase, inhibition of acetylcholinesterase results in an increase in acetylcholine contents [9]. Increased level of latter has been shown to enhance the secretion of catecholamine in fish [10], which may bring about glycogenolysis A significant ($p<0.05$) elevation of blood glucose level in the fish exposed to Dimethoate has been observed in the present study. Our results are supported by the previous workers who have reported increase in the blood glucose level in different fish subjected to pesticides. Ceron *et al.,* [11] observed an increase in blood glucose level of *Anguilla anguilla* exposed to Diazinon. Sastry and Sharma [12] also reported hyperglycemia in the fish, *Ophiocephalas punctatus* exposed to Diazinon.

Change in blood glucose has been suggested as useful general indicator of stress in teleost. Nemcsok and Bores [13] reported that blood glucose appeared to be sensitive indicator of environmental stress in fish. The stress related hyperglycemia reported in many species of teleosts is mediated mainly by the effects of catecholamines on glucose release from the liver, the main carbohydrate store in fish, with epinephrine being more potent then nor epinephrine [14]. Chan and Woo [15] noted that cortisol has shown to promote catabolism of peripheral tissues via increased gluconeogenesis leading to hyperglycemia. In the present study increase in the serum glucose level and reduction in the muscle and liver glycogen after pesticide exposure may be due to mobilization of glycogen reserves [9]. It is well established fact that the glycogen is a stored form of energy which can be easily mobilized for energy production. Further the rapid secretion of glucocorticoids [16] and catecholamines [17] from the adrenal tissue after the exposure to toxicant enhance the glycolysis which results in elevation of plasma glucose. Both these hormones are known to produce hyperglycemia in animals. Therefore, the hyperglycemia condition occurred in experimental fishes in the present study may be attributed to enhance break down of energy reserves (glycogen) in different tissues of toxicant exposed fish brought about by hyper secretion of aforesaid hormones.

Proteins are important organic substances required in tissue building and repair. Under extreme stress conditions they are also a source of energy [18]. In the present study a significant ($p<0.01$) decrease in the protein content was observed due to Dimethoate exposure. Umminger [19] observed decrease in the protein content in the fish *Fundalus beterolitus* and stated that the aquatic inhabitants exposed to toxic conditions utilized protein as energy source. Saxena *et al.,* [20] recorded the reduction in protein content in *Channa punctatus* due to Melathion toxicity. The possible reason for protein depletion in the present study may be due to stress conditions in the experimental fish due to pesticide toxication. During stress conditions fish needed more energy to detoxify the toxicants and to overcome stress. Since fish have a very little amount of carbohydrates, the next alternative source of energy is protein to meet the increased energy demand. The depletion in protein fraction in liver and muscle tissues may have been due to their degradation and possible utilization of degraded proteins for metabolic purposes.

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