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DIMETHOATE INDUCED ALTERATIONS IN CARBOHYDRATE METABOLISM OF COMMON CARP, *Cyprinus carpio* (LINN.)

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ABSTRACT

This study was conducted to assess the sub lethal effects of dimethoate on carbohydrate metabolism of common carp, *Cyprinus carpio.* In the short term (96 hr) and long term (36 day) study, healthy juveniles of common carp were exposed to 0.96 mgl^{-1} (60% of LC_{50}) and 0.48 mgl^{-1} (30% of LC_{50}) of dimethoate respectively and a parallel control was run simultaneously. Significant decrease in glycogen levels of liver and muscle was noticed in both short as well as long term test. Glucose levels in blood registered significant increase and remained significantly higher than control in both 96 hr and 36 day exposure. Decrease in glycogen levels showed dependence both on exposure concentration and duration. This study demonstrated that carbohydrate metabolism can be adversely affected by dimethoate both in short as well as long term sub lethal exposure.

KEYWORDS: *Cyprinus carpio,* Dimethoate, LC_{50}, Carbohydrate, Glycogen, Glucose

Indiscriminate use of chemicals in agriculture, household settings and health and hygiene programmes is a threat to the ecology and stability of aquatic ecosytem. Variety of more or less persistent chemicals released in the environment ultimately find their way to aquatic ecosystems and cause water pollution (Pereira et al., 1996). Among agrochemicals, synthetic pesticides especially organochlorines and organophosphates constitute an important group of aquatic pollutants. Accumulation of these pesticides in aquatic ecosystems results in adverse effect on health of non target organisms including fish (Velmurugan *et al*., 2007; Singh *et al*., 2009).

Dimethoate, [IUPAC Name – O, O dimethyl S - (N methylcarbamoylmethyl) phosphoro-dithioate],CAS No.60-51-5, popular as rogor is a systemic organophosphate insecticidewidely used for controlling insect pests in agriculture and horticulture.. Like other organophosphates it also works as nerve poison and inhibits acetylcholinesterase in synapses and neuromuscular junctions (Cope *et al.* 2004). It is very selective as insecticide because relative rates of degradative enzymes namely esterases and amidases is relatively slower in insects than in mammals (Rose and Hodgson, 2004).

It is non – photodegradable, undergoes very slow hydrolysis and shows moderate persistence in water. Persistence of dimethoate in water is pH dependent being greater in acidic environment. Dimethoate is acutely toxic and classified as possible human carcinogen by USEPA based on tumor occurrence in mice. In the WHO acute hazard ranking dimethoate is rated as moderately hazardous. The United States Environment Protection Agency (USEPA) in its interim re registration eligibility decision for dimethoate has declared 0.43/0.84 mg/l as the no observed adverse effect concentration (NOAEC) and the lowest observed adverse effect concentration (LOAEC) for technical grade dimethoate in fish.

Biochemical profile of aquatic inhabitants including fish is affected by toxic chemicals reaching into water bodies. Alteration in biochemical parameters of fish can, therefore, be used as a reliable and early indicator of deterioration in the health of water body. Many workers have reported deterioration in biochemical indices of fish after pesticide exposure (Singh et al. 2004; Begum, 2008; Tilak et al., 2009; Suneetha, 2012; Singh et al., 2015). But scanty reports are available on effect of dimethoate on carbohydrate metabolism in fish. Therefore, this study attempts to assess the effect of dimethoate on carbohydrate metabolism of common carp, a very popular food fish available afresh round the year.

Common carp, *Cyprinus carpio* is a highly palatable fish introduced in India in the year 1957. It is a good fish for culture due to its hardy nature, omnivorous habit, fast growth rate and easy breeding in confined water. As a result, this exotic carp has now become common in ponds, reservoirs, and river systems of northern India and makes substantial proportion of the inland capture and culture fishery.

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MATERIALS AND METHODS

Fish Collection and Acclimatization

Fish were brought from ponds of Uttar Pradesh state government's local hatchery (Bhojpur, Sultanpur) and treated with 0.05% potassium permanganate for two minutes. They were acclimatized to laboratory conditions for two weeks into plastic pools of 500 liter capacity. Fishes were fed ad lib rice bran mixed with mustard oilcake in the ratio of 2:1, and three-fourth of the pool water was renewed daily during acclimatization.

Water Quality Parameters

The experiment was conducted under natural photoperiod and temperature in the month of September. The temperature of the experimental water was 23 ± 1.5^{0}C, pH was 7.2 ± 0.4, Dissolved oxygen was 7.2 ± 0.6 mg/l, free carbon dioxide was 6.2 ± 0.4 mg/l and total hardness as calcium carbonate was 112 ± 3.2 mg/l.

Preparation of Stock Solution and Exposure of Fish

The 96 hr LC_{50} value of dimethoate for common carp fingerlings was determined as 1.60 mg/l by Finney's probit method (Singh *et al.* 2009). Based on 96 hr LC_{50} value, a sub lethal concentration of 0.96 mg/l (60% of LC_{50}) and 0.48 mg/l(30% 0f LC_{50})dimethoate was selected for the experiment. Technical grade dimethoate (ROGOR 30% EC, Rallis India Ltd, Mumbai) was procured and stock solution (1mg/ml) was prepared in absolute alcohol. Glass troughs of 30 liter capacity were filled with 25 liter of water in which 24 ml and 12 ml of stock solution in short and long term test respectively was mixed thoroughly before releasing the fishes. In control trough the commensurate absolute alcohol quantity was mixed in 25 liter of water. Healthy individuals of common carp (age, five months, size, 15-20 cm, and weight, 90-150 gm) were sorted and separated in groups of six fish each irrespective of sex from the acclimatized stock. Feeding was stopped 24 h before the experiment and was not resumed during the course of the experiment. Six fish were released in each test and control trough. No mortality occurred in test and control groups during the experiment. At the 24, 48, 72 and 96 h exposure in short term test and at 6, 12, 24 and 36 days in long term test fishes were sacrificed for collecting samples for analysis.

SAMPLE COLLECTION AND ANAYSIS

Fish were first immobilized in ice and then dissected out carefully so as to expose their heart for collection of blood through a syringe from conus arteriosus. Immediately after collecting blood, a drop of blood was touched with the test strip of Accu Chek glucometer for determination of blood glucose level.

Part of the liver and muscle were removed and processed for estimating glycogen content. Tissues were homogenized in 10 ml of 10% TCA and centrifuged; filtrate was processed for glycogen estimation after Van der Vies (1954) and optical density taken within two hours, at 650 nm on digital spectrophotometer of MS Electronics, India (Model, 305).Standard curve was plotted using 0.1 mg/ml glucose as standard solution. Optical densities of tissue samples were converted into concentration with the help of standard curve, which were subsequently used for determining glycogen in mg/g of wet weight of tissues. Results obtained were analyzed by student's t test to test the significance (< 0.05).

RESULTS AND DISCUSSION

The biochemical effects of dimethoate exposure on blood glucose levels and glycogen levels in liver and muscle along with their statistical significance are summarized in Table 1 and plotted in Fig 1, 2 and 3.In the beginning of exposure, glucose level increases sharplythen gradually declines but remains significantly higher ($p<0.050$) than control throughout theexposure in both short and long term test. Glycogen levels of liver and muscle both exhibit significant declineat all observations of short and long term dimethoate exposure but decline is sharper in liver as compared to muscle. The decline in glycogen though statistically significant at all duration of exposure, it is maximal at 96 hours in short term and 36 days in long term.

Table 1: Changes in blood glucose, liver and muscle glycogen levels of *Cyprinus carpio* during dimethoate exposure

Conc. (dimethoate) Mg/l	Duration day	Blood Glucose Mg/dl		Liver Glycogen Mg/g wet wt		Muscle glycogen Mg/g wet wt	
		Control	Test	Control	Test	Control	Test
0.96	1	65.36±4.82	142.24±6.83	68.98±6.64	48.62±3.34	8.02±0.65	6.12±0.59
0.96	2	65.80±6.36	133.54±5.57	67.64±5.88	42.44±4.25	8.01±0.38	5.48±0.29
0.96	3	67.32±4.44	122.48±3.26	68.22±2.82	32.67±3.98	8.11±0.45	4.94±0.47
0.96	4	66.84±2.66	106.62±6.24	67.94±4.23	24.42±2.72	8.05±0.34	4.47±0.35
0.48	6	66.0±3.08	128.66±5.64	67.88±3.42	48.98±2.66	8.0±0.34	6.42±0.14
0.48	12	67.68±2.30	124.66±3.68	64.58±2.29	42.84±2.62	7.88±0.20	7.08±0.21
0.48	24	63.66±3.62	124.16±3.64	60.24±2.78	38.69±3.85	7.10±0.35	5.98±0.46
0.48	36	64.16±2.65	106.48±6.72	55.11±2.41	33.86±2.84	6.93±0.44	5.45±0.24

Values are mean ± sd of six observations; all test values are significant ($p<0.05$)

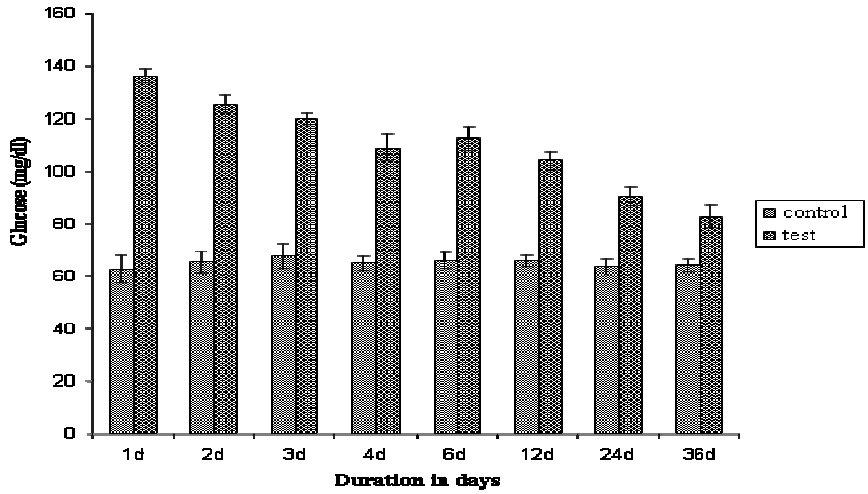

Figure 1: Changes in blood glucose levels of *Cyprinus carpio* exposed to dimethoate [Exposure concentration: 0.96 mg/l in short term (day 1 - 4), 0.48 mg/l in long term (day 6 - 36)]

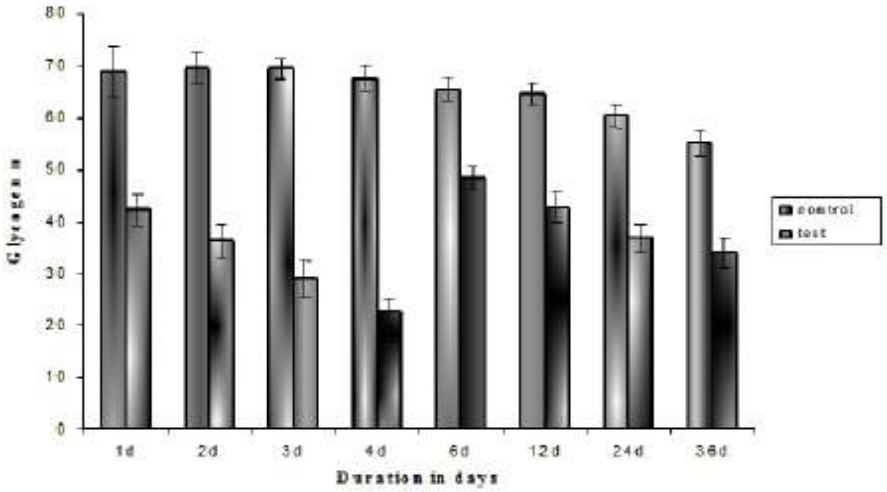

Figure 2: Changes in liver glycogen levels of *Cyprinus carpio* exposed to dimethoate [Exposure concentration: 0.96 mg/l in short term (day 1 - 4), 0.48 mg/l in long term (day 6 - 36)]

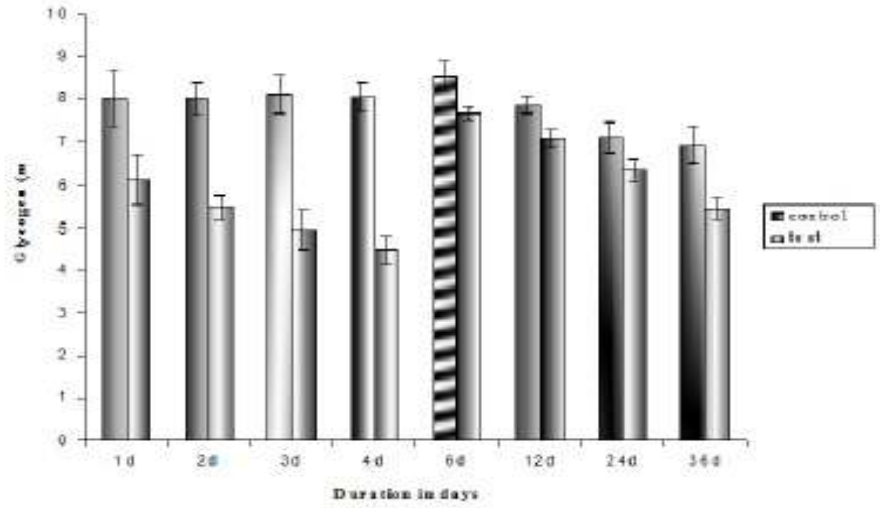

Figure 3: Changes in muscle glycogen levels of *Cyprinus carpio* exposed to dimethoate [Exposure concentration: 0.96 mg/l in short term (day 1 - 4), 0.48 mg/l in long term (day 6 - 36)]

Significant increase in blood glucose observed in the present study is in agreements with findings of Singh and Srivastava, (1982, 1995) and Singh *et al.* (1997) in *Heteropneustes fossilis,* Okechukwu Ogueji and Auta (2007) in *Clarias gariepinus,* Velisek *et al.* (2009) in *Cyprinus carpio*, exposed to pesticides. Similarly decrease in muscle and liver glycogen has also been reported by many workers in fish after exposure to different organophosphate pesticides. Decrease in glycogen level have been reported in *Colisa fasciatus* after exposure to malathion and carbaryl (Singh *et al.* 2004); and in *Channa punctatus* after exposure to dimethoate (Tripathi *et al.* 2003);in *Heteropneustes fossilis*after exposure to malathion (Singh and Srivastava, 1993) and propoxur (Singh *et al.* 1997); in *Glossogobius giuris* after exposure to malathion (Venkataramana *et al*. 2006); in *Mystus vittatus* after exposure to sumidon (Neethirajan and Mathavan, 2004); and in *Catla catla, Labeo rohita and Cirrhinus mrigala* after exposure to chlorpyrifos (Tilak *et al*. 2005).

Decline in tissue glycogen levels along with hyperglycemia may be the result of stimulation of catecholamine secretion by dimethoate, an organophosphate. Organophosphates are acetylcholinesterase inhibitors and lead to accumulation of acetylcholine which in turn has been found to increase catecholamine secretion in *Gadus morhua* by Nilsson *et al.* (1976). Increased secretion of catecholamine brings about glycogenolysis and hyperglycemia through raised level of cyclic AMP (Terrier and Perrier, 1975). In fact a variety of stressors are known to stimulate the adrenal tissue, resulting in increased level of circulating catecholamines (Nakano and Tomlinson, 1967). Both of these groups of enzymes bring about glycogenolysis and produce hyperglycemia. Increased level of blood glucose is considered as a general response of fish to acute and sub lethal pollutant exposure. (Verma *et al*. 1983; Ceron *et al*. 1997; Luskova *et al*. 2002). Wedemeyer *etal.* (1981) have observed thatphysical and chemical stress induce disorders in carbohydrate metabolism reflected in the form of hyperglycemia..

Carbohydrates are the primary and immediate source of energy. Under stress conditions energy demand increases and carbohydrate reserves are first to register depletion (Arasta *et al*. 1996). Decrease in glycogen levels of liver and muscle along with concurrent hyperglycemia indicates disturbance in the carbohydrate metabolism of the exposed fish. A fall in glycogen levels in pesticide exposed fish indicates its rapid breakdown (glycogenolysis) to meet the enhanced energy needs under conditions of stress. Under dimethoate toxicity anaerobic metabolism appears to become more important as a result of hypoxia, occurring due to reduced oxygen consumption. Singh et al. (2009) have reported reduced oxygen consumption in *Cyprinus carpio* exposed to dimethoate. Shift toward anaerobic metabolism is also proved by decrease in the enzyme levels of succinate dehydrogenase and malate dehydrogenase, the Krebs' cycle enzymes (Rao and Rao, 1986; Neethirajan and Mathavan, 2004). Trend towards anaerobic metabolism is also indicated by increased level of lactate dehydrogenase (LDH), which has been reported by Balint *et al*. (1995) in

common carp and Tripathi *et al.* (2003) in *Channa punctatus* after pesticide exposure.

This study demonstrates that dimethoate adversely affects carbohydrate metabolism causing hyperglycemia and reduced glycogen levels in liver and muscles of fish which may be detrimental health of fishes.

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