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Effect of Methyl Parathion on the Carbohydrate Metabolism of the Fish, *Cirrhinus Mrigala*

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Abstract: In general, any stress inducing substance will affect the respiratory metabolism of an animal. Any alteration in the intermediary metabolism due to stress is bound to affect the activity of oxidative enzymes like lactic dehydrogenase (LDH) and succinic dehydrogenase (SDH). Both enzymes are involved in carbohydrate metabolism and have been used as an indicative criterion of exposure to chemical stress. Several authors have reported that the disturbance in the oxidative metabolism which leads to an alteration in whole oxygen consumption in different species of fishes exposed to pesticides. Carbohydrates are the primary and immediate source of energy. In stressed condition, the carbohydrate reserve (glycogen) is depleted to meet the energy demand. Depletion of glycogen may be due to the direct utilization for energy generation, a demand caused by the pesticide induced hypoxia. Similar findings have been reported in frog *Rana tigrina*. Glycogenolysis seems to be the result of increased secretion of catecholamines due to stress. Pesticides also inhibit energy production by suppressing aerobic oxidation of carbohydrates leading to energy crisis in animals. LDH and SDH are widely used in toxicology and clinical chemistry to diagnose the cell, tissue and organ damage. In the present study, the toxicological effect of methyl parathion on the LDH and SDH activity has been made in the fish, *Cirrihinus mrigala.* The study revealed that the acute toxicity (TU_a) of methyl parathion was 14 ppm.

Key words: LDH, SDH, methyl parathion, glycogen, *Cirrihinus mrigala*, acute toxicity.

1. Introduction

The indiscriminate use of pesticides to boost agriculture production has affected the ichthyfaunna either directly or indirectly. Increased use of chemical pesticides results into the excess inflow of toxic chemicals mainly into the aquatic ecosystem [1, 2]. The aquatic flora and fauna are affected by the toxic substances which eventually enter into systems or bring about external damage [3-5]. Several species of fish are susceptible to deleterious effects when exposed to heavy metals, pesticides and other environmental stressors [6-8]. Monitoring of blood parameters both, cellular and non-cellular may have considerable diagnostic value in assessing early warning signs of pesticides poisoning.

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Blood is a patho-physiological reflector of the whole body. Knowledge of the physiological action of the toxicant helps to predict on important sublethal effects and analysis of biochemistry, hematology and histopathology may be used to determine the mode of action of the toxicant. In recent years, biochemical variables were used more when clinical diagnosis of the fish physiology was applied to determine the effects of external stressors and toxic substances. Therefore, biochemical evaluations are gradually becoming a routine practice for determining the health status in fish.

In general, any stress inducing substances will affect the respiratory metabolism of fish. Any alteration in the intermediary metabolism due to stress is bound to affect the activity of oxidative enzymes like LDH and SDH. Nevertheless, the LDH is an important glycolytic enzyme which is present in

almost all the kinds of tissues [8]. This enzyme is involved in carbohydrate metabolism and has been used as an indicative criterion of exposure to chemical stress [9] and the alteration in the normal LDH activity pattern was found to be the oxygen stress after exposure [10]. LDH is a parameter widely used in toxicology and clinical chemistry to diagnose the cell, tissue and organ damage. However, the potential of this enzyme as an indicative criterion in toxicity tests in fishes has scarcely been explored. SDH is the vital enzyme of the Krebs cycle which catalyses succinate to fumarate. The decrease in SDH would affect the conversion of succinate to fumarate and might cause a block in the Krebs cycle. The decreased activity in the SDH in the liver following the treatment of methyl parathion has been reported by Ravishankar et al [11]; Kumari and Sinha [12, 13]. Therefore, the toxicity of methyl parathion (organophosphate) on the oxidative enzymes (LDH & SDH) in the fish, *Cirrhinus mrigala* has been studied to evaluate the effect of methyl parathion on the carbohydrate metabolism.

2. Materials and Methods

Cirrhinus mrigala, commonly known as "Naini", a common carp obtained from the local hatchery. Fishes were acclimated to laboratory conditions for about a week. They were kept in aquarium tank (250 L) and the water was constantly aerated by a static system. During the acclimation period, they were given artificial (commercial) feed and ground shrimps available in the local market to avoid the possible effects of starvation on any parameters under study. The chemical characteristics of the aquaria water were measured. Short term test of acute toxicity over a period of 96 h were performed on the fishes following renewal of bioassay. Fishes were exposed intracoelomatically with 1/3rd of LC_{50} of the pesticide methyl parathion. After 24, 48, 72 and 96 h of exposure, fishes were sacrificed for different assays.

The behavior and conditions of the fishes were noted every 24 h up to 96 h. The fishes which failed to respond even to strong tactile stimuli were considered dead and removed from the aquarium.

2.1 Determination of LC_{50}

The experiments were repeated for several times and only arithmetic mean of the experiments at each concentration was taken to express the results. LC_{50} values were determined by EPA-Probit analysis programme [14].

2.2 Blood Collection

The fishes were taken out of the aquarium individually through fish net with a minimum possible disturbance. After preliminary investigations, the blood samples were collected from caudal fin dissection as described by many authors. In the present study, the blood collection from the caudal fin dissection had to be abandoned because there was an unusual elevation in the LDH and CPK activities which were recorded due to leakage from the surrounding muscle tissues. Thus cardiac sampling was the only suitable method available as an alternative to obtain blood under the present study. After the blood sampling, the liver tissues were taken for enzymatic assays.

2.3 Liver

Liver was taken out and soaked with filter paper and subsequently analyzed for SDH activity. Prior to assay, the tissue was properly homogenized in an electrical homogenizer and centrifuged at 4 °C in a Remi Refrigerated Centrifuge (Model C-30).

2.4 SDH Activity

To estimate the activity of SDH in liver, a known amount of liver was homogenized in a known volume of phosphate buffer (pH 7.4). Succinate dehydrogenase activity of the homogenate was measured following the method of Kun and Abood [15]. The assay mixture in the experimental tube contained 0.25 mL of 0.1 M Na-succinate, 0.25 mL of 0.3% aqueous triphenyl tetrazolium chloride and 0.5

mL of 5% tissue homogenate. A blank without homogenate and a control without substrate were also run simultaneously. All the tubes were incubated at 33 °C for a period of 1 h. The red coloured formazan formed during the incubation period was extracted with 5 mL acetone and optical density was measured at 580 nm in Shimadzu UV-Vis Spectrophotometer Model-1800, the enzyme activity was expressed as μg formazan per hour.

2.5 LDH in Blood

10 μl of serum was taken and LDH activity was measured by Kit method according to the method of Elliot and Wilkinson [16].

The principle of the method is that an equimolar amount of nicotinamide adenine dinucleotide hydrogen (NADH) is oxidized to NAD^+ during the reduction of pyruvate in the presence of LDH. The oxidation of NADH results in the decrease in absorbance at 340 nm.

The decrease of absorbance at 340 nm is directly proportional to LDH activity in sample, where one unit of LDH activity is defined as the amount of enzyme which catalyzes the formation of 1 μ mol/L of NAD^+ per minute under the condition of the assay.

$$\text{Pyruvate} + \text{NADH} \xleftrightarrow{\text{LDH}} \text{Lactate} + \text{NAD}^+$$

The sample and reagent were mixed and absorbance was recorded after 1 min. The absorbance was recorded after 1, 2, and 3 min at 340 nm to get the change in absorbance per unit time.

Glucose was determined by GOD-POD method.

2.5.1 Principle

Glucose is oxidized by glucose-oxidase (GOD) to produce gluconate and hydrogen peroxide. The hydrogen peroxide is then oxidatively coupled with 4-amino-antipyrene (4-AAP) and phenol in the presence of peroxidase (POD) to yield a red quinoenine dye that was measured at 505 nm in Shimadzu UV-Vis Spectrophotometer Model-1800. The absorbance at 505 nm is proportional to the concentration of glucose in the sample.

Table 1 The reaction conditions.

Parameter	Value
Temp. (°C)	30
Sample (μL)	10
Reagent-1 (μg)	500

Table 2 Composition of reagent 1.

Parameter	Value
Glucose oxidase (μL)	2,000 μL
Peroxidase (μL)	1,200
4-AAP (mmol/L)	0.246

Table 3 Addition of reagents follows the order.

Particulars	Blank (μL)	Standard (μL)	Sample (μL)
Reagent-1	1,000	1,000	1,000
Distilled water	10	-	-
Reagent-2	-	10	-
Sample	-	-	10

Reagent 2: glucose standard 100 mg/dL.

$$\text{Glucose} + 2H_2O + O_2 \xleftrightarrow{\text{GOD}} \text{Glucose} + H_2O_2$$

$$2H_2O_2 + \text{4-AAD} + \text{Phenol} \xleftrightarrow{\text{POD}} \text{Quinoemine dye}$$

Absorbance of the coloured solution was directly proportional to glucose concentration when measured at 505 nm.

2.5.2 Procedure

One reagent blank and one standard are sufficient for each series.

It was mixed well and incubated for 15 min at room temperature and then measured the absorbance of standard and sample against the reagent blank at 505 nm.

2.5.3 Calculation

Glucose concentration in the sample was calculated using the following formula:

$$\text{Glucose} = \frac{\text{Abs. of the sample}}{\text{Abs. of standard}} \times \text{Concentration of standard (mg/dl)}$$

3. Results and Discussion

In the present investigation, it has been observed that the LC_{50} was 14 ppm of methyl parathion in the fish, *Cirrihinus mrigala*. The effect of sub lethal concentration of pesticide, i.e., 1/3rd of LC_{50} was studied in the fish till 96 h. The results revealed that

there was a sudden significant increase ($p < 0.002$) in 24 h ($p < 0.001$) and in 48 h LDH activity in the serum and thereafter significant decrease ($p < 0.03$) in 72 h. The results also revealed that there was a concurrent increase in blood glucose level in 24 h and thereafter sudden decrease in 72 h. On the contrary, the aerobic enzyme SDH decreased significantly ($p < 0.003$) during 24 h and significant increase ($p < 0.006$) in 96 h.

Table 1 shows that following the treatment of methyl parathion there is a significant increase in LDH activity during 24 and 48 h with significant enhanced increase of glucose in 24 h suggesting that the fish suffers from oxygen stress. Relative to the pre exposure (control), there is a decreased activity of SDH during 24 and 48 h (Table 1). The increase in LDH activity with simultaneous increase in glucose concentration in blood (Table 1, Fig. 1) is indicative of increased rate of glycolysis. The levels of blood glucose are considered to be the index of metabolic homeostasis in all vertebrates. Nakano and Tomhson [17] reported that all types of stress increased the secretion of catecholamines which in turn increased the breakdown of glycogen and enhanced the blood sugar level. The blood sugar has a direct relationship with metabolism [18] so the fluctuations observed in blood sugar level in the present study could be attributed to the differences in rate of respiration and activity.

The pollutants are known to alter the physiological and biochemical state of animal by inducing marked changes in the activities of several enzymes [19]. In the present study, mitochondrial oxidative enzymes were found to be altered and inhibited after methyl parathion exposure. The decreased activity of SDH and increased activity of LDH (Fig. 2) affects the energy synthesizing machinery of the cells. These changes appeared to favour a less efficient anaerobic metabolism probably due to the inability of tissues in the treated fishes to derive sufficient oxygen for normal metabolic functions. Therefore, it is suggested that methyl parathion may have induced toxicity in target organs. Nevertheless, it triggered the anaerobic metabolism and arrested the Krebs cycle as it is evident from the decrease in SDH activity (Table 1, Fig. 2). The general manifestation of stress is called "General Adaptation Syndrome" which is divided in 3 stages:

The alarm reaction which occurs before adaptation has occurred (1st stage);

The stage of resistance in which adaptation is optimal (2nd stage);

The stage of exhaustion in which acquired adaptation is lost (3rd stage).

The adaptation is mediated through HPA axis [20].

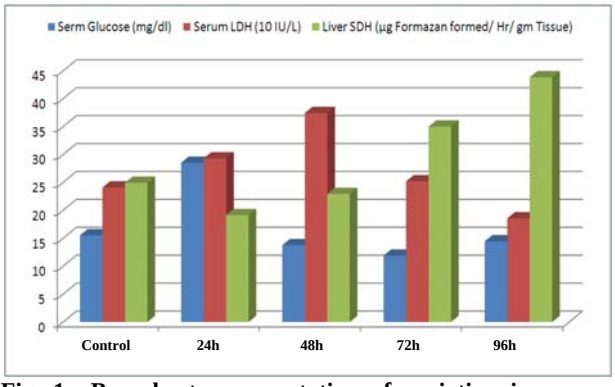

Fig. 1 Bar chart representation of variation in serum glucose, serum LDH and liver SDH as a function of time.

Table 4 Variation in serum glucose, serum LDH and liver SDH as a function of time.

Parameter (serum)	Control	Time duration			
		24 h	48 h	72 h	96 h
Glucose (mg/dL)	15.53 ± 4.178	28.54 ± 8.54 $P < 0.03$	13.78 ± 0.95 $P < 0.417$	11.90 ± 2.49 $P < 0.045$	14.462 ± 1.95 $P < 0.563$
LDH (IU/L)	241.0 ± 21.43	293 ± 39.64 $P << 0.002$	374 ± 53.72 $P < 0.016$	252 ± 15.362 $P < 0.878$	185 ± 4.95 $P < 0.207$
Parameter (tissue)					
SDH (liver) (µg formazan formed/hr/gm of tissue)	24.94 ± 3.31	19.17 ± 4.50 $P < 0.126$	22.950 ± 4.623 $P < 0.097$	35.0 ± 3.69 $P < 0.010$	43.83 ± 2.403 $P < 0.039$

Table 5 Physicochemical charecteristics of the aquaria water.

Parameter	Value
Temperature (°C)	28
pH	8.2
Dissolved oxygen (mg/L)	8.4
Electrical conductivity (μs/cm)	310
Total hardness (mg/L)	284

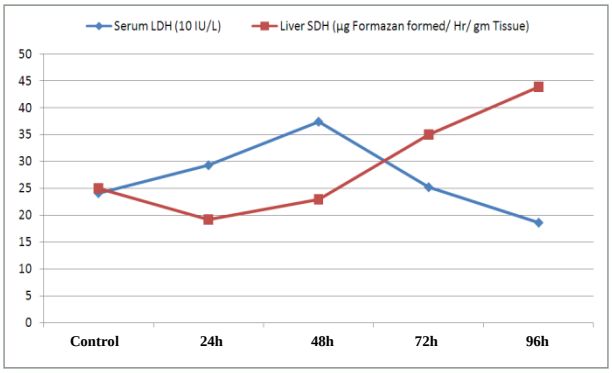

Fig. 2 Relationship between serum LDH and liver SDH as a function of time.

The initial elevation of the LDH activity (Table 1, Fig. 1) could be explained in terms of oxidative metabolism during the initial hours of exposure, as a result of sudden response to toxic stimulus of the pesticide. With the onset of symptoms the rate decreased in 72 h probably due to acclimation to the toxic environment (2nd stage of adaptation syndrome). Thereafter, there was a decrease in LDH activity (3rd stage of adaptation syndrome where acquired adaptation was lost as the fish switched out towards normalcy). Biphasic alterations were observed in the increase and decrease of LDH and SDH activities, respectively. Initially, the decrease in SDH activity in 24 h and 48 h (Fig. 2) suggests that methyl parathion inhibits the conversion of succinate or inhibits the oxidation process in the TCA cycle. The inhibition of SDH activity may be related to alterations in the enzyme and substrate levels. Initial decrement in the affinity between enzyme and substrate followed by enzyme substrate complex formation for SDH during the pesticide treatment has been reported by Gupta [21]. Singer et al. [22] believed that the activity of SDH was controlled by the metabolic state of mitochondria. Camba and Dianzami [23] observed inhibition of SDH activity in methyl parathion exposed animals and opined that the inhibition of SDH may be due to the action of parathion on mitochondria presumably in its membrane. The inhibition of SDH activity initially may be due to the same reason assigned by Camba and Dianzami [23].

The increased activity of LDH (Fig. 2) suggests that the rate of glycolysis is much higher in methyl parathion exposed fishes (Table 5). The unequivocal decreased SDH activity in the liver of the fishes (Table 5, Fig. 2) indicates the prevalence of anaerobic metabolism in the pesticides exposed fish. Hence, the results have been discussed in relation to the shift in respiration metabolism from anaerobiosis to aerobiosis.

4. Conclusions

Liver being the organ for interconversion and storage of food stuffs and centre for all detoxification mechanisms, the energy demands for it are much more. Drastic shift of LDH and SDH activities of this organ in the fish indicate towards high energy demands to bring about metabolic co-ordination and for activation of detoxification mechanism. Blood is a pathophysiological reflector of the whole body. It is often debated whether methyl parathion act through the inhibition of acetylcholine esterase (AchE) or affecting the target organs directly. Based on the observations in the present study, the respiratory distress occurred earlier to the neurotoxic effects such as that the fish slowly became lethargic and disrupted shoaling. It was observed that the fishes in the aquarium became restless and started surfacing and gulping air immediately following the treatment of methyl parathion. Therefore, it is suggested that the first molecule to be affected is the Hb molecule and then neurotoxicological behaviour through inhibition of AchE.

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