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BIOCHEMICAL ALTERATIONS CAUSED BY METHYL PARATHION IN FRESH WATER FISH *CLARIAS BATRACHUS* (LINN.)

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ABSTRACT – Test fish *Clarias batrachus* procured from different localities of Modinagar reared in the laboratory and allowed to acclimatized for about 10-15 days. Prior to the blood sampling through cardial vein or cardiac puncture, fish were anaesthetized using standardized Tricaine mesylate. Subsequently, the values of biochemical parameters were measured using semi autoanalyser and test kits. Biochemical composition (Total protein = TP, Albumin = Al, Globulin - Gl, Serum Glutamate oxaloacetate transminase = SGOT and Serum qlutamate pyruvate transminase = SGPT) were investigated with the time of exposure to methyl parathion, which show fluctuations in fish physiological and histological dysfunction.

Key words : Biochemical, fresh water fish, *Clarias batrachus,* anaesthesia, methyl parathion.

INTRODUCTION

Use of pesticides for control of diseases in agriculture and aquaculture have increased enormously during the last few decades. The ultimate sink for these pesticides is the basically aquatic environment. The surface run off from the agricultural lands carry the pesticides in the aquatic ecosystem, which enter into the organisms through food webs and also through contact in water (Edwards, 1973; Brown, 1978; Murty, 1986). The toxicity may be either acute in lethal or chronic to fish in sublethal concentrations. Among these two, the later contractions are slow poisons disturbing the biochemistry of the organisms (Rand and Petrocelli, 1985) and infact more dangerous due to the use of harmful chemicals.

Pesticides affect the survival, growth rate, fecundity and reproductive activity of fish (Hirose, 1975; Park *et al,* 2004; Singh and Singh, 2006). Toxicants even in very low concentration have been reported to interfere with basal metabolism and suppress the reproduction (Kondal *et al*, 1989), steroidogenesis (Wester and Vos, 1994; Singh and Canario, 2004; Sehgal and Goswami, 2005), lipid metabolism (Lal and Singh, 1987; Singh, 1992; Singh and Singh, 1992; Singh and Kime, 1994), gonadotropin levels (Van Der Kraak *et al*, 1992; Singh *et al*, 1994) and also act as reproductive biomarkers (Sepulveda *et al*, 2004).

Certain metals changes the reduction in protein, lipid and cholesterol contents of the tissues lead to retardation of growth, and longevity of the organisms (Ram *et al,* 1984). The analysis of biochemical constituents like proteins, lipids and carbohydrates has been done in various concerned organs of fish (Dasgupta and Sircar, 1985). Once toxic substances enter into the body, they damage and weaken the mechanism concerned leading to the physiological, pathological and biochemical disorders (Astra *et al*, 1995; Mulley *et al*, 1996; Arasta *et al*, 1999).

Numerous biochemical indices of stress have been proposed to assess the health of non-target organisms exposed to toxic chemical in aquatic ecosystem (Nimmi, 1990). Chronic stresses have developed in fish due to fluctuations in pH, dissolved oxygen and unionized ammonia in water (Das *et al*, 1994). Changes in plasma enzyme activity are used as indicators of tissue injury, environmental stress, or a diseased condition. The variation in enzyme activities in the freshwater fish exposed to various pollutants have been reported (Begum *et al*, 1995; DeSmet *et al*, 2001; Humtsoe *et al*, 2007).

The accumulation of toxic metabolites, like ammonia is one of the important factor limiting stocking densities in ponds (Smith, 1972). The unionized form of ammonia (NH_3) (UIA) is far more toxic to fish than ionized form (Downing and Merkens, 1955). Acute toxicity studies have been conducted by Colt and Tchobanoglous (1976), Robinette (1976), Thurston *et al* (1978 and in India by Kumar and Arya, 1995, Kumar and Muni, 1997.

The toxicants reaching the aquatic ecosystem get enriched the aquatic food chain through bio-accumulation, bioconcentration and bio-magnification process (Murty, 1986). Fish protein is one of the main source of energy, which play an important role in maintenance of blood glucose (Jrueqer, 1968). The heavy metals and chemicals cause the great threat to the health of Indian aquatic ecosystem (Joshi *et al*, 2002).

Untreated community wastes, pesticides as well as dumping of organic and inorganic wastes from industries into water resources have been become a great challenge to aquatic fauna especially fish (Lokhande and Kelker, 1999). Toxic effect of pesticides in fish at cellular level particularly in liver, kidney and other vital organs have been observed causes of gastrological and neuro muscular damage to the organisms feeding upon them (Wagh and Khillare, 1989).

MATERIALS AND METHODS

Clarias batrachus Linn. 1758 (family - Clariidae) locally known as mangur ranging from 24 cm to 28 cm in length and 145 g to 170 g weight were collected from local fish resources and acclimatized in laboratory for 10-15 days under natural photoperiod. Fish were fed with commercially fish food and water was changed in every alternate day. Physiochemical characteristics of water were analysed using the method of APHA. Water has temperature of 27° ± 2°C, pH 7.6 ± 1.5 and O2 6.5 ± 1.0.

To determine LC_{50} value of the fish different doses of methyl parathion were given to the 4 groups (having 10 fish each) to fish by using formula of brown (9) in glass aquaria of 80 lt. capacity.

60 fish were divided in 6 groups (10 each) 4 experimental and 2 control. Before giving the chemical, feeding of experimental fish were stopped 24 hrs. before. In this manner blood sampling were made of 3, 7, 15, and 30th days.

For blood sampling experimental fish was anaesthetized by using Tricaine mesylate (Tricaine methanesulfonate, TMS, MS-222) and blood was taken by from either caudal vein or cardiac puncture by using heparinized syringe. Plasma was obtained by centrifugation 3500 rpm for 20 minutes and then placed in sterile tubes for biochemical analysis.

For the analysis of total proteins, albumin and globulin test kits of Span Diagnostics Ltd., Surat, India and for Serum Glutamate oxalo acetate transminase and Serum qlutamate pyruvate transminase test kits of Agappe Diagnostics Ltd., Kerala, India were used.

To ensure adequate quality control two level controls were run. It is recommended that each laboratory establishes its own frequency of control determination. Value obtained with control material should fall within a specific range. It should be noted that the use of quality control material checks performance of complete system that comprises of machine (Analyser), material (Reagents) and fish (Procedure).

RESULT AND DISCUSSION

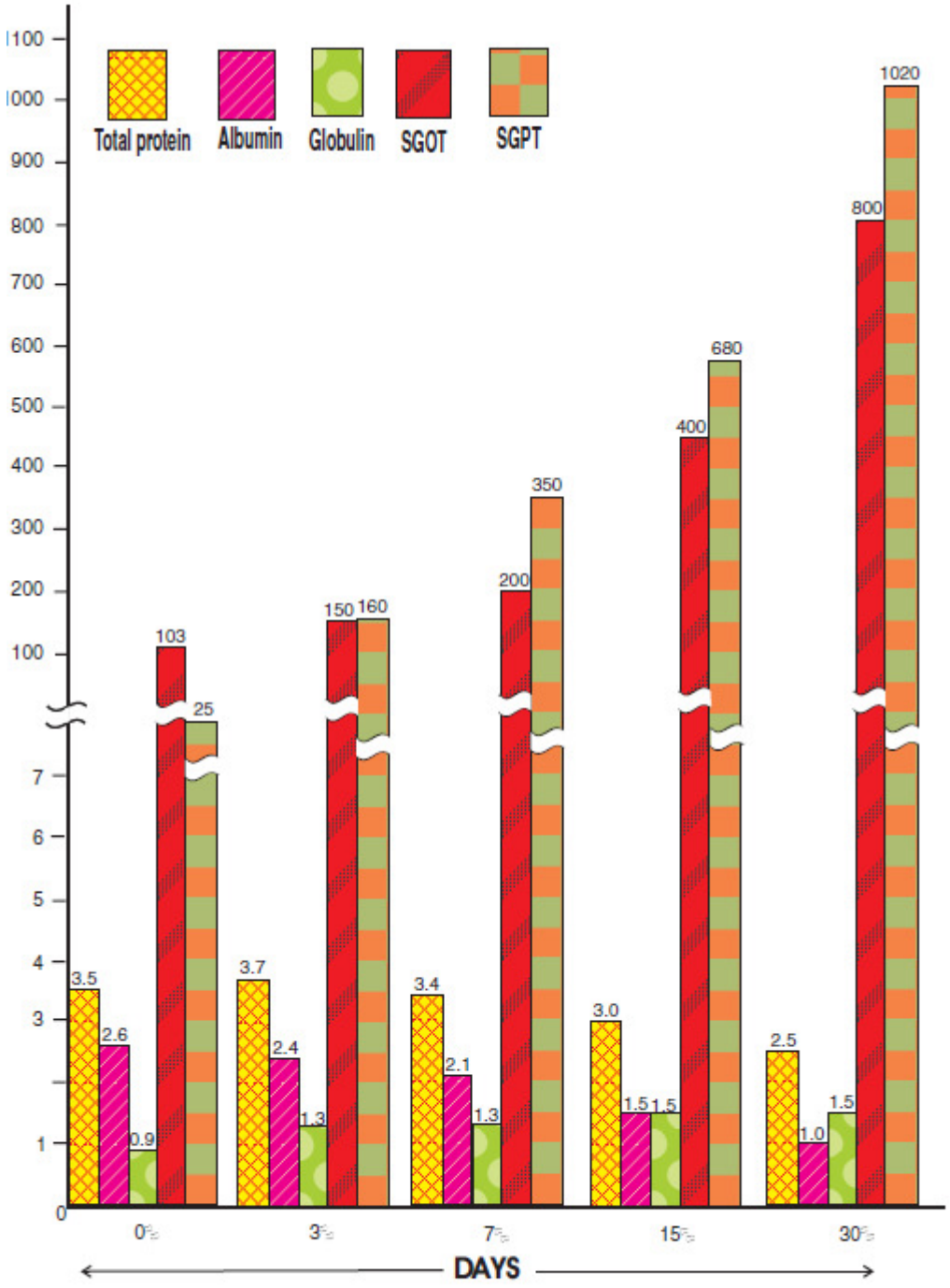
The variations/fluctuations in the above biochemical substances in fish *Clarias batrachus* exposed to sublethal concentration of methyl parathion i.e.96.4 µg/lt for different periods show alarming conditions for fish and the organisms feeding upon them especially human being. These variations indicate towards the continuous decay of different tissues and muscles also, where the pesticide is accumulated.

Change in Total Protein and Albumin

Albumin and the globulin are the main constituents of protein in which only albumin is left if globulin is separated (Corcoran, 1977). Amount of total protein is little bit increased initially but then after it continuously decreased which indicate towards the severe non viral liver cell damage and also towards the damaging kidney as the total protein loss is occurring through it (Gendler *et al*, 1984). As the albumin is digested into the amino acids and then transported into the blood to face the starvation.

Table 1: Different value of various biochemical constituents at different interval under chemical stress.

	Control	Experimental			
Days / Constituents	0	3	7	15	30
Total protein	3.5	3.7	3.4	3.0	2.5
Albumin	2.6	2.4	2.1	1.5	1.0
Globulin	0.9	1.3	1.3	1.5	1.5
SGOT	103	150	200	450	800
SGPT	25	160	350	680	1020



Scale: Upto 7 cm. 1.0 cm = 10 µg/lt.
Then after 1.0 cm = 100 µg/lt.

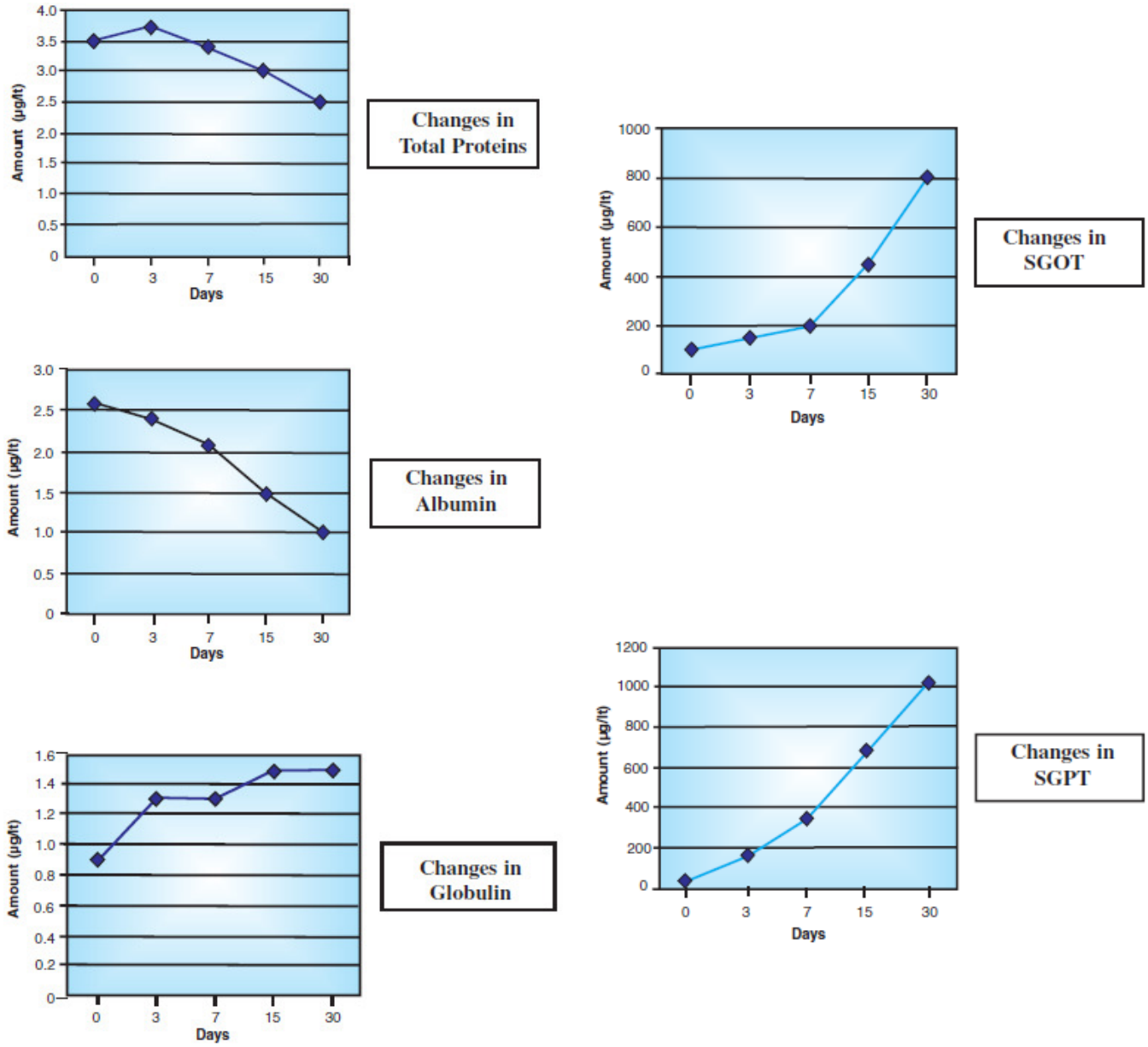
Value of different biochemical constituents are given on the top of broken bar in µg/lt.

In this sense the continuous decrease in the albumin content in the fish body show the presence of lethargic substances in its environment (Johnson *et al*, 1999) it can not tolerate the absence of food for a long time due to which the longevity of life is effected.

Changes in Serum Glutamate Oxalo Acetate Transminase (SGOT)

It is present in most of the tissues, especially in cardiac muscle, liver cell, skeletal muscle and kidneys. Injury to these tissues results in the release of the enzyme in blood steam.

Increased levels are found in cardiac problems. The duration and extent of increase is related to the infarct. GOT determination is of considerable value to differentiate other cardiac disorders.



Changes in Serum Glutamate Pyruvate Transminase (SGPT)

It is present in most of the tissue, but mainly found in the liver. Increased levels are found in hepatitis, cirrhosis, obstructive jaundice and other hepatic diseases. Serum qlutamate pyruvate transminase activity is markedly elevated even before clinical signs of jaundice become apparent in diseases associated with hepatic necrosis. Slight elevations are also found in cardiac disorder.

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