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EFFECT OF METHYL PARATHION (AN ORGANOPHOSPHATE) ON ELECTROPHORETIC PATTERNS OF PROTEINS IN GILL AND MUSCLE OF FRESH WATER CAT FISH *HETEROPNEUSTES FOSSILIS* (BLOCH)

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ABSTRACT

The present study was carried out to determine the effect of Methyl parathion (an organophosphate) on electrophoretic patterns of proteins in the gill and muscle of freshwater cat fish *H.fossilis* (Bloch). The fish were exposed to 2% sub lethal concentration of pesticide Methyl parathion at different time intervals i.e. 24 h; 48 h; 72 h and 96 h for a period of 10 days. The changes in the tissue proteins of vital organs such as gill and muscle were examined on 7.5% of SDS –PAGE. The protein patterns indicated that the muscle has higher number of protein bands compared to gill and control. The number of protein bands observed in gill of test fishes were found to be lesser than the muscle control. The protein banding patterns were identified by standard marker protein and Rm values were calculated accordingly. The results of the present study of electrophoretogram of both gill and muscle showed homology in protein bands with minor variations.

KEYWORDS: Protein patterns, Methyl parathion, SDS –PAGE, *H. fossilis.*

INTRODUCTION

Pesticide usage is a critical concern because it has an adverse effect on the delicate ecosystem. The transfer of the pesticides to the aquatic ecosystem creates a need to fully understand their effect in the resident biota.[1] In many areas the sensitive ecosystems are at risk because of point source runoff pesticides from agricultural and urban sources to aquatic ecosystems affecting aquatic biota.[2,3] Pesticide pollution severely affects aquatic organisms and organisms at high tropic levels including human beings through food chain[4,5] These

pesticides absorb rapidly through different routes and accumulated in tissues like liver, kidney and fat and leads to many physiological and biochemical changes there by influencing the activities of several enzymes and metabolites and finally causes the disturbance of entire metabolic processes.[6-9]

Many pesticides have been reported to produce a number of biochemical changes in fish and at lethal concentrations, pesticides have been attributed to enzyme and endocrinal activity as chemoregulators in fish.[9] This may lead to provide an early warning signal to stressed organism. The source of these parameters is an indicator responding to environmental effects and can also serve as a marker for the xenobiotic exposure.[10,11]

In the present investigation an attempt has been made to study the effects of Methyl parathion (an organophosphate) on electrphoretic patterns of proteins in gill and muscle of fresh water cat fish *H. fossilis* through SDS-PAGE.

MATERIALS AND METHODS

Collection of Samples and preparation of OP compound

Adult fishes (weighed about 50-70gms) were collected from local fresh water tanks within the radius of 15km from the laboratory by the netting with the help of local fisher men. They were immediately brought to the laboratory and introduced into a plastic bucket (30X30X60cm) and disinfected with Potassium permanganate and washed thoroughly prior to introduction of fish (to prevent fungal infection). The fishes were acclimatized for about one week in aquaria they were fed with commercial fish food daily until their use. The sub lethal concentrations of the pesticide Methyl parathion (2% E.C) were prepared in 95% Acetone to yield a concentration of 100mg/ ml which were further diluted with distilled water to get a working solution prepared by the procedure.[12] In the present investigation sub lethal concentration of 0.01, 0.04, 0.06 µg of pesticide were taken for 24h,48h,72h and 96 h exposure. A control batch corresponding to each test group was simultaneously experimented to compare the toxicated effect of Methyl parathion in various tissues.

Preparation of Samples for study

At the end of each exposure period fishes were sacrificed, the tissues such as gill and muscle were dissected out and were used for the further analysis. The tissues were weighed to nearest milligram and were homogenized in 0.01M Tris HCl buffer (pH 7.5) containing 0.9% NaCl. The concentration of tissue homogenates varied from tissue to tissue. The tissues after

homogenation were placed in ice jacketed centrifuge tubes. The extracts were centrifuged at 2000rpm for 10min in a clinical centrifuge at room temperature. The supernatant were mixed with equal volume of 20% sucrose solution containing 0.5% bromophenol blue as tracking dye, An aliquot of 0.1ml of this mixture was used for loading the sample on to the gel for electrophoretic separation of protein patterns.

SDS-PAGE Analysis

Homogenates (10%) of gill and muscle were prepared in Tris-HCl buffer (pH 7.2) and centrifuged at 10,000 rpm for 10min. The pellet was washed with chilled acetone and was dissolved in sample buffer 2ml of 0.5M Tris HCl (pH 6.8), 40% glycerol (1-6ml), 10% SDS (3.2ml), 2-mercaptoethanol (0.8ml), 0.1% (W/V) bromophenol blue (0.4 ml) and heated at 95^0c for 1min.

Experimental procedure for preparation of SDS-PAGE

The supernatants were mixed with equal volumes of 20% sucrose containing 0.1% SDS, β-mercaptoethanol and bromophenol blue was used as the tracking dye. An aliquot of 0.1ml (5mg) of the tissue extract was loaded on to the separating gel directly. The electrode buffer 0.025M tris and 0.192M Glycine was used for according to standard procedure[13] whereas 0.074 M Tris, 0.1% SDS adjusted to pH 7.8 with con. HCl. A constant current of 50 volts for the first 15 min followed by 150 volts for the rest of the run was applied to the gel. The current supply was terminated when the tracking dye migrated to a distance of 8.0 cm from the origin.

Staining Procedure and standardization of protein bands

A solvent containing 0.25% Coomassie brilliant blue in methanol, water & acetic acid (5:5:1) was used for staining the proteins separated on gel by using standard method.[14] The molecular weight standards used in comparing the variations noticed in the SDS-PAGE, were of Low molecular weight protein standards (15 to 100 KDa) from the SIGMA-Chemical company (USA).

RESULTS AND DISCUSSION

The relative mobilities of the protein patterns in different tissues of the fresh water cat fish *H. fossilis* exposed to Methyl parathion for 10days are presented in (Tab. 1,2 and 3 and fig. 1,2 and 3) respectively. The protein patterns observed on SDS-PAGE stained with Coomassie brilliant blue indicated distinct differences in the mobility of some bands of the fish tissues.

Comparison of the protein bands of various regions with standard protein marker revealed that the variation is higher in the regions of slow moving zone-A and those with fast moving zone-C and the patterns obtained in the middle region-B were more or less similar in different tissues of fish.

The electrophoretogram obtained in (Tab.1 and fig.1) reveals that there is a decrease in the intensity of protein bands of gill compared to control at different time intervals, under the Methyl parathion exposure at different time intervals of the tissue samples, the gill protein patterns observed have shown more decreased intensity in banding pattern compared to control. The Rm values of protein subunits were 0.23, 0.41, 0.48 (Zone-A nearer to molecular marker 100- 70 KDa) at 24 h and some with Rm value 0.21 and 0.38 were observed at 48 h. The protein band intensity was completely absent at 72 h and 96 h in fish gill (Zone-A). The Rm values of protein bands (Zone-B 55-35 KDa) with 0.53, 0.55, 0.61, 0.65 at 24 h, 48 h, 72 h and 96 h were observed respectively in tissue of gill.

The protein band of Rm value in fish muscle was 0.26 which is observed at 24 h and at 48h intensity of protein band was decreased. The Rm value of protein band was 0.33 observed only at 48h, but at 24h and 72 h protein band was completely disappeared. Protein band with Rm value of 0.40 and 0.46 were observed at 48 h and 24 h respectively. At 72 h and 96 h the band was completely absent. Two protein bands with Rm values of 0.46 and 0.55 were observed at 24 h only, while a band with Rm value of 0.50 was observed at 72 h only. The Rm value of protein band 0.55 was observed at 24 h only. It was interesting to note that remaining time intervals i.e., 48 h, 72 h and 96 h, the protein bands were completely absent.

Table 1: Rm Values of Gill proteins of *H.fossilis.*

Standard Protein Marker	Control	24H	48H	72H	96H
	0.01				
			0.21		
0.23	**0.23**	0.23			
0.38			0.38		
	0.41	0.41			
0.48	**0.48**	0.48			
			0.50	0.50	
	0.53		0.53		0.53
		0.55			
	0.58				
	0.61	0.61			
0.65		0. 65	0.65	0.65	
0.70		0.70	0.70		

		0.73			
		0.76			
0.83	**0.83**		0.83	0.83	0.83
0.86		0.86			
	1.0				

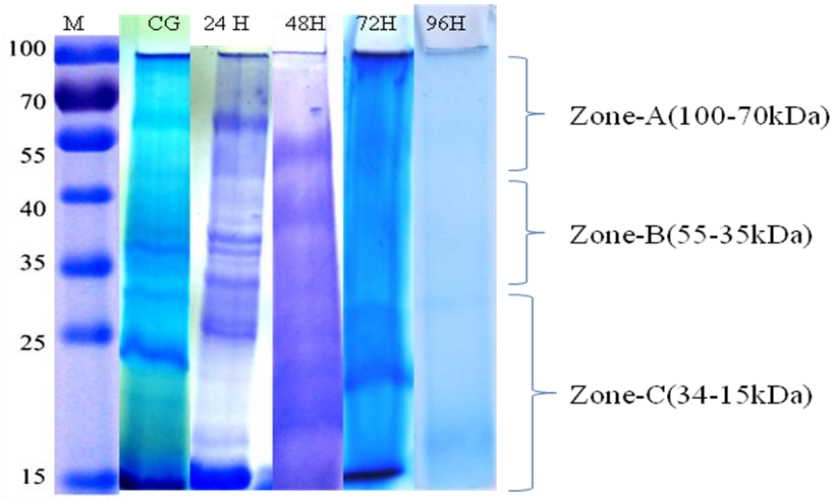

Fig. 1 Gill expressed Protein bands in Different time intervals after Organophosphate exposure

Table 2: Rm Values of Muscle proteins of *H.fossilis*.

Marker	Control	24H	48H	72H	96H
	0.05				
	0.06	0.06			
	0.16				0.16
	0.21				
0.23					
		0.26	0.26		
			0.33		
0.38		0.38			
			0.40		
		0.46			
0.48					
			0.50	0.50	
				0.53	
		0.55			
0.66	**0.66**		0.66	0.66	
0.70		0.70			
			0.73		
	0.76	0.76		0.76	
	0.78				
	0.80	0.80	0.80	0.80	
0.83					
0.86	**0.86**	0.86		0.86	
	0.91		0.91		
		0.96	0.96		

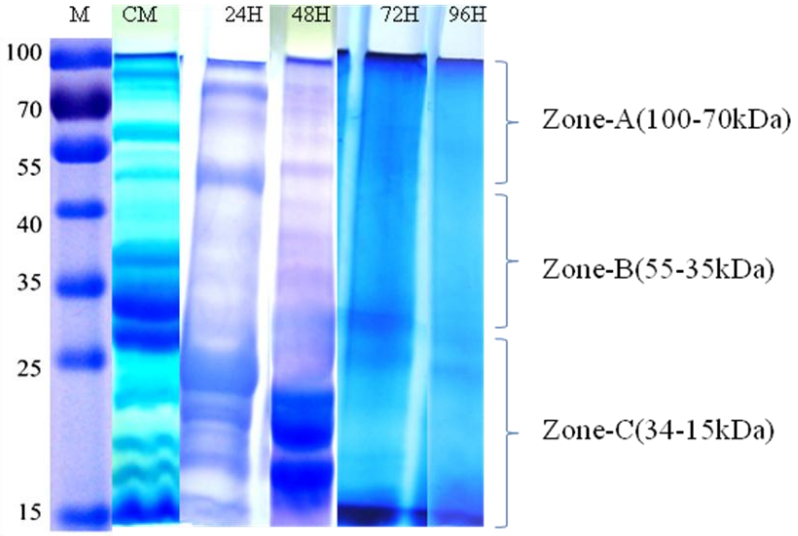

Fig.2 Muscle expressed Protein bands in Different time intervals after Organophosphate exposure

The protein patterns found in the fish muscle were as follows:

A band of protein with Rm value 0.66 was observed at 48 h and 72 h, and its intensity was completely decreased compared with control. A protein band with Rm value 0.70 was observed at 24 h, while completely absent at 48 h,72 h and 96 h. The protein band of Rm value 0.73 was observed at 48 h only. A protein band of Rm value 0.76 was observed at both 48 h and 72 h. A protein band with Rm value (Zone-C fast moving zone mol. Weight 34-15KDa) 0.78 was completely absent at all time intervals. A protein band with Rm value 0.86 was observed at 24 h, 48 h and 72 h, where as not found at 96 h. A protein band of Rm value 0.86 were observed at 24 h and 72 h and the band intensity was found to be decreased when compared with control. A band with high intensity and Rm value 0.96 was observed at both 24 h and 48 h and with more intensity at 48 h. A protein band with low intensity of Rm value 1.0 was observed at 24 h only and was not present at 48 h, 72 h and 96 h. It was interesting to note that the protein showed more decrease in band intensity (or) significant fading when compared to control. The variations in banding patterns of protein subunits due to change in the turnover of (synthesis or degradation) various proteins.

Pesticides may inhibit the expression of some genes (or) may activate the others to produce specific mRNAs, which may subsequently be translated into specific proteins called stress induced protein.[15-18] An alteration of protein metabolism was observed in fish exposed to different types of environmental stress like metals and pesticides.[19] It was reported that when cytoplasmic protein of liver and the skeletal muscle of *Clarias batracus* exposed to Endosulphon and Methyl parathion for 1 to 28 days, the appearance of new proteins after

exposure to pesticide was found which was a clear alternation in the cytoplasmic proteins.[20,22,23]

In the present study, SDS-PAGE performed for the tissues of gill and muscle of *H. fossilis* exposed to Methyl parathion, the protein patterns of pesticide exposed tissues showed decreased in intensity and some protein bands were completely disappeared.

Many authors have reported similar observations, found the decrease in the protein sub units induced by Endosulphon and Fenvalrate in fresh water fish *Labeo rohita* through SDS-PAGE.[24] The impact of Acetamiprid toxicity on electrphoretic patterns in liver, brain and gill tissues of fresh water fish *Oreochromis massambicus*[25-26] had observed by using new techniques such as electrphoretic studies on the muscle proteins of three species of genus *Puntius* (Osteichthyes-Cyprinidae).

The protein subunits showed a steady decreasing trend in intensity of all the fractions throughout the exposure period demonstrating an inhibitory effect of Endosulphon on kidney and muscle LDH.[27,28] have observed, that slight reduction (or) decrease in intensity of proteins in Diazinon treated fish Nile thilapia, which indicates that these proteins were highly affected the stress caused by the pesticide.[29] have demonstrated that Malathion showed profound effect on protein patterns of *H. fossilis* and found new protein bands and some others disappeared after treatment.

In the present study we report that the effect of Methyl parathion on electrophoretic patterns of proteins in gill and muscle of fresh water cat fish *H. fossilis*, when exposed to pesticide the protein subunits showed decrease in intensity and some protein subunits have completely disappeared. The electrophoretogram of both gill and muscle showed homology in protein bands with minor variations.

CONCLUSION

Thus, Present study has concluded that the long term exposure of Methyl parathion becomes a continuous health hazard for the fish population. Therefore it is required to monitor the aquatic system and predict the toxic effect of pesticides on fish.

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