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MALATHION INDUCED CHANGES IN LACTATE DEHYDROGENASE ISOENZYMES OF RED MUSCLE OF *Heteropneustes fossilis* (BLOCH)

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Malathion, an organophosphate pesticide, is generally used in crop fields to reduce the loss of farm produce. However, its concomitant run-off from agricultural fields poses major threat to the aquatic fauna and especially fishes are exposed to such toxicity causing changes in various biochemical parameters involved in different metabolic pathways. The five isoenzymes of LDH identified electrophoretically, show conspicuous changes in terms of number and quantity with respect to different concentrations and exposure periods of malathion.

Keywords: Malathion; lactate dehydrogenase; isoenzyme; red muscle; inhibition.

1. INTRODUCTION

Problems of pollution in relation to fishes and their environment have received increasing attention for a long time. Many investigations have been made to measure the toxicity of pollutants on aquatic organisms [1]. These endeavors have heralded new technique establishing fish as a tool in toxicity testing of pollutants in aquatic systems.

The run-off of such xenobiotics is not only restricted to aquatic system rather all those who are linked to the fish community through food chain are indirectly exposed to them eventually causing threat to survival of such species [2] They are carried to the mankind in a subtle manner to bring about wide range of implications on them.

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Malathion {O,O-dimethyl -5-1, 2-di-(Ethoxycarbamyl) ethyl phosphorodithioate} also known as carbophos, maldison and mercaptothion is an organophosphate pesticide used to control mosquitoes, household insects, animal ectoparasites etc besides application in crop fields.

Many scientists have worked on LC50 values for 24h, 48h, 72h, 96h etc. on different fishes to evaluate the lethality of malathion and to find out relevant dosages for exposure to specific experimental species. Sublethal concentrations at different exposure periods have been determined by many workers [3-7] and have also investigated the effect of malathion on biochemical and hematological parameters of different fishes.

The inhibitory effect of malathion on acetylcholine esterase (AChE) has been widely studied to ascertain the neurotoxic effect of this pesticide [8,9]. Effect of endosulfan on LDH has also decreases LDH activity [10].

The present work has included the effect of this pesticide on a respiratory enzyme Lactate Dehydrogenase (LDH) by evaluating qualitative and quantitative changes of isoenzymes for assessing shift of respiratory pathways of an air-breathing catfish *Heteropneustes fossilis*. Isoenzymes: LDH_1, LDH_2, LDH_3, LDH_4 & LDH_5 have been electrophoretically separated and assessed changes to provide vital information about the adaptability of this fish.

2. MATERIALS AND METHODS

Irrespective of sex, live adult size specimens of *H. fossilis* of equal weight (30±2 gm) and length (20±2cm) of one stock were stocked and fed in cemented fish tanks (10'x10'x10') for a period of 10 days before commencement of different sets of experiments in order to acclimatize them in new environment.

The 96h LC-50 value of malathion for *H. fossilis* determined by Dutta et al. [3,4] was considered as reference value for conducting the experiment. The two concentrations: 5% (C_1) and 10% (C_2) concentrations of LC-50 value of malathion were used to conduct the experiment. Similarly two exposure periods: 20 days after treatment (20 DAT) and 40 days after treatment (40 *DAT*) were considered as two exposure periods to evaluate the changes in muscle LDH isoenzyme pattern and their quantities as well.

The 96h LC-50 value of malathion for *H. fossilis* was found to be 11.798 ppm [3,4]). The two concentrations C_1 0.60 ppm and C_2 1.2 ppm of commercial grade of malathion (cynamid India Ltd.) were set up for exposure of fishes in two aquaria of 40L along with a third aquarium 40L for control group.

Thus three experimental sets each containing 20 fishes of similar length weight and sex were kept in three aquaria of 40L capacity. Six fishes from each treatment group were sacrificed on 20 DAT and 40 DAT after exposure of this pollutant along with the control group.

Red Muscle (RM) from the base of the pectoral fin was taken out after the sacrifice of the fishes of each set. Homogenates were prepared in a chilled 0.01 M Tris-HCl buffer of pH 7.5 (Smith 1976). The homogenates were subjected to refrigerated centrifugation at 2000 rpm for 15 minutes. The supernatants thus obtained were poured off and were kept in separate vials inside the freezer (-10°C) for biochemical analysis.

The electrophoretic separation of LDH was done on 7% Polyacrylamide Gel Electrophoresis (PAGE) by the method described by Smith [11]. The LDH was estimated by following the protocol using 0.1 M Sodium L- Lactate 5ml with Nicotinamide Adenine Dinucleotide (NAD+), Nitroblue Tetrazolium Salt (NBT), Phenazyne Methosulphate, 0.2 M TrisHCl buffer (pH 8.0) 10 ml and glass distil water 35ml.

Sodium L-Lactate substrate was prepared by reaction of sodium carbonate (6.07g) and DL-Lactic acid (10.6 ml) and the solution was diluted to 250ml.

Incubation time: Gels were placed in freshly prepared reaction mixture for 15 minutes at 37° C when the bright blue bands of LDH Isoenzymes appeared. Gels were fixed and preserved in alcoholic gel wash.

Zymogram of LDH isoenzymes was prepared on graph and scanning of gels was done to evaluate the isoenzyme activity.

The quantitative estimation of LDH isoenzymes activity (µg/mg/hr) was estimated after scanning of gels under a densitometer with subsequent photography of isoenzyme bands. Zymogram was also drawn subsequently with respect to isoenzymes band activities and their Rf values.

3. RESULTS AND DISCUSSION

Effect of malathion on LDH was studied in terms of its changes in isoenzyme number and activity. Control sample of RM possessed all five Isoenzymes designated as LDH_1, LDH_2, LDH_3, LDH_4 & LDH_5 at

Rf 0.15, 0.20, 0.25, 0.28 & 0.32 respectively. Of all five isoenzymes the LDH_5 was the most prominent one having the highest activity: (Figs. 1 and 2).

When *H. fossilis* was exposed to malathion, the LDH isoenzymes decreased in their activities. Still all the five isoenzymes were observed on 20DAT of both the concentrations of malathion. However, the increase in exposure period on 40DAT led to the disappearance of four isoenzymes at Rm 0.15, 0.20, 0.25 & 0.28 at both concentrations (C_1 & C_2) of this pollutant. The persisting LDH_5 also lost its activity to great extent and its lowest value was observed at C_2 on 40 DAT of this pollutant.

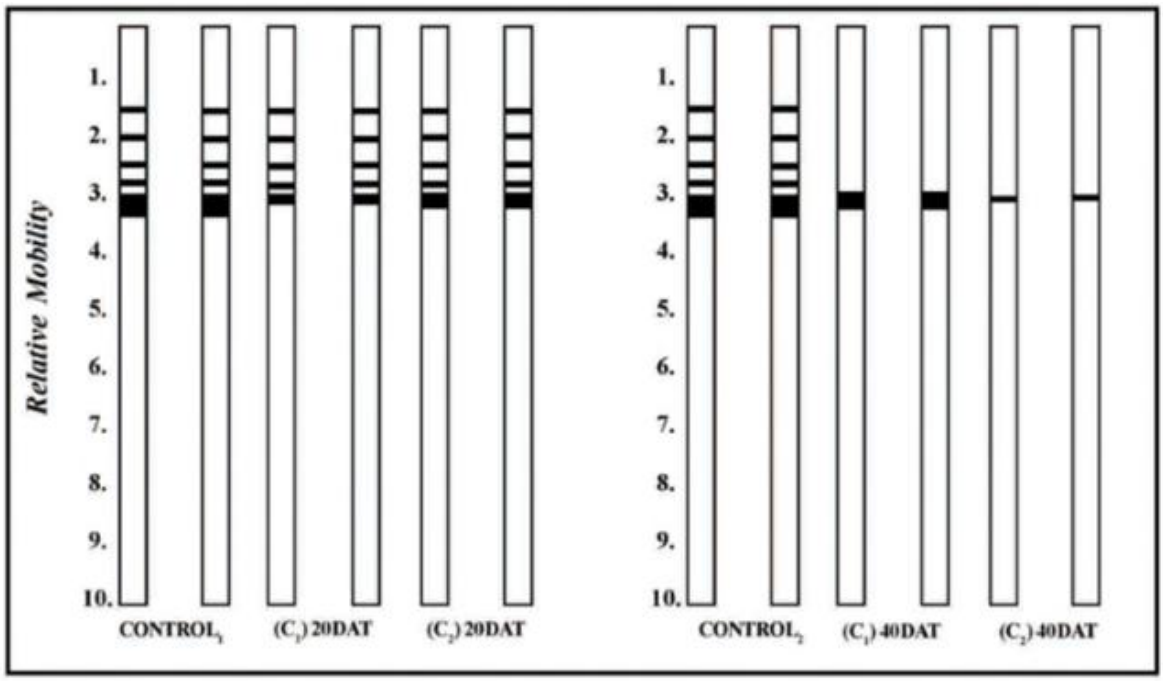

Fig. 1. Zymogram showing changes in Lactate Dehyrogenase (LDH) isoenzyme pattern of red muscle of H. fossils exposed to Malathion

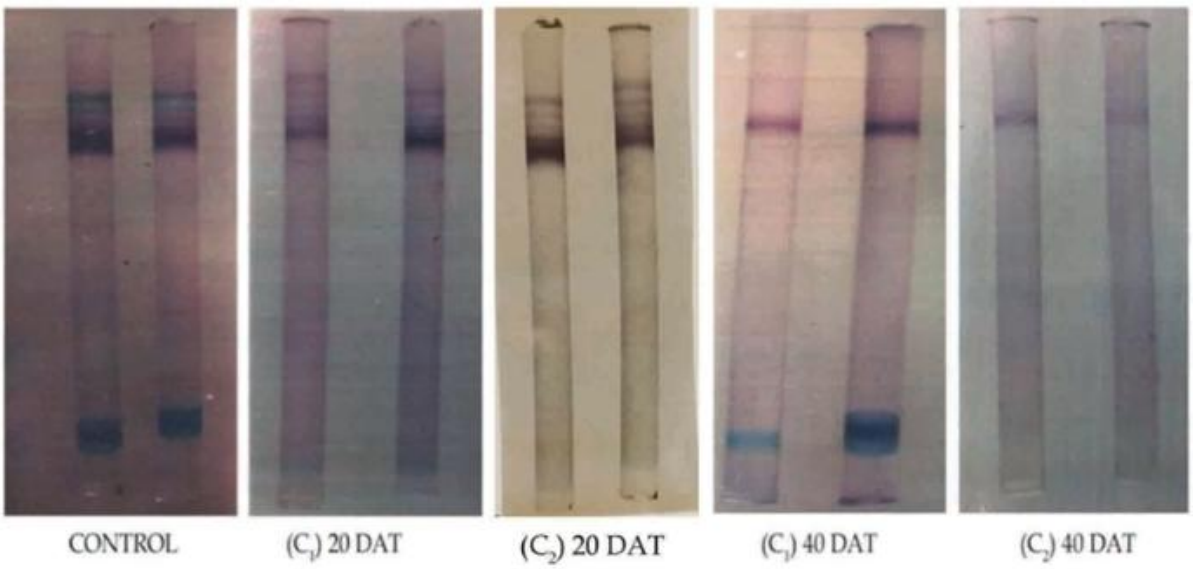

Fig. 2. Gels showing LDH isoenzymes in control & treated groups

Table 1. Changes in Lactate Dehydrogenase (LDH) Isoenzymes Activities(µg/mg/hr) in Red Muscle of *H. fossilis* exposed to malathion

Isoenzymes	I	II	III	IV	V
Rm	0.15	0.20	0.25	0.28	0.32
Control $_1$	30.033 ±1.4023	33.8800 ±0.6079	38.0133 ±1.5246	39.4333 ±1.2350	148.3500 ±2.0942
C_1 (20 DAT)	20.8200 ±0.3578	24.6900 ±0.8779	45.9283 ±2.1523	48.1833 ±0.4377	63.2367 ±1.0162
C_2 (20 DAT)	9.5833 ±1.0999	16.1367 ±0.8978	17.1683 ±1.1665	17.3667 ±0.9415	96.4983 ±1.0854
Control $_2$	28.6500 ±1.0999	32.3000 ±1.6025	39.3433 ±1.0012	40.7100 ±1.3428	151.0500 ±1.2855
C_1 (40 DAT)	Nil	Nil	Nil	Nil	89.9700 ±1.8705
C_2 (40 DAT)	Nil	Nil	Nil	Nil	44.7700 ±0.8272

C-Concentration DAT- Days After Treatment
Values are Mean± S.E. of six samples

The LDH isoenzymes differ significantly in maximum velocity Vmax and Michaelis constant Km for their substrate Pyruvate. LDH_5, a predominant isoenzyme of muscle favors rapid reduction of very low concentration of Pyruvate to Lactate in skeletal muscle whereas isoenzyme LDH_1 (H4) favors rapid oxidation of Lactate to Pyruvate in heart [12], and therefore predominant in heart muscle [13]. Thus the LDH_1 and LDH_5 show metabolic differences between two tissues [14]. The increase in LDH_5 at C_2 20 DAT shows a tendency of recovery of the fish at this stage to withstand the increased concentration of the pollutant.

Decreased LDH_1 and other three isoenzymes activities might have increased lactic acid with corresponding decrease in pyruvic acid putting a hindrance in oxidative pathway of respiration, a factor explaining sluggish nature of fish in toxic environment. Decreased enzyme activity under malathion toxicity may suggest a transient shift from aerobic to anaerobic metabolism [15,16]. This shift suggests that limited supply of energy is still ensured during pesticide's toxic stress for the sustenance of life. The 40 DAT exposure in case of this pollutants resulted in complete disappearance of all isoenzymes except LDH_5. Thus the ATP supply is ensured by this sole isoenzyme even at extreme toxicity and exposure in order to provide a chance for survival and race continuation.

4. CONCLUSION

The adverse effects of pesticides may not be observed overtly in an animal by mere observation of its behavioural changes as sometimes it may appear quite normal, however, the pollutants toxicity might have badly affected its physiology to the extent to threaten its survival. The changes in LDH isoenzyme pattern on exposure of fish to the pollutant can be proved as diagnostic tool for assessing the respiratory distress of the animals.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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