Research Article

Evaluation of LDH Activity on Exposure to Profenofos and Carbosulfan by Using Nondenaturing Polyacrylamide Gel Electrophoresis (Native PAGE)

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ABSTRACT

Lactate dehydrogenase converts that lactate to pyruvate and has very important role in carbohydrate metabolism. LDH activity depends on its isoenzymes and it has 5 isoenzymes and the activity changes under pathological conditions. The increase in LDH activity suggests the increased anaerobic conditions, exposure periods, favor anaerobic respiration to meet the energy demands, where aerobic oxidation is lowered. All bands in profenofos and carbosulfan treated tissues showed decrease (lane 5-6) in intensity when compared to control. The difference of the distribution of LDH isoenzymes in tissues is known to reflect difference in their metabolic activity.

INTRODUCTION

Pesticides were found to adversely affect a number of biological functions, thus causing harm to the non-target organisms. Organophosphorus and carbamate compounds are known for this persistence in the environment and accumulation in the tissues for long periods for controlling the loss of produce due to pest attack and as a consequence of the demand for producing more food, there has been an increasing use of pesticides in our country. Although it may yield immediate benefits, the use of pesticides in the long run, is brought with many dangers[1,10].

Lactate dehydrogenase catalyses the oxidation of lactate and reduction of pyruvate during anaerobic glycolysis. It is a tetrameric molecule consists of two separate loci which codes for A and B subunits of this enzyme. The A and B subunits indiscriminately associate and form five tetrameric isoenzymes (A4, ABB1, A2B2, A1B3 and B4) [2], isoenzymes are multiple forms of a single enzyme, which often have different isoelectric points and therefore can be separated by electrophoresis. Electrophoretic studies were done extensively on the different tissues of various animals from which it reveals that the enzyme exhibit in multi molecular forms and functions [3]. LDH electrophoretic patterns could help in investigating and to locating the pesticide stress. Stress reflects on respiratory metabolism as LDH is a key enzyme in carbohydrate metabolism and occurs virtually in all tissues. It is indicative of variation in tissue functioning as a toxicant[4].

LDH enzyme in fish has always been the subject of much attention [5,10]. It is to be noted that, not much information was available with the changes in the expression of LDH on toxicity with profenofos and carbosulfan. The aim of this work was to evaluate the expression of LDH isoenzymes in different tissues of fish *Labeo rohita* after exposure to sublethal concentrations of profenofos and carbosulfan for 15 days using native gel electrophoresis.

MATERIALS AND METHODS

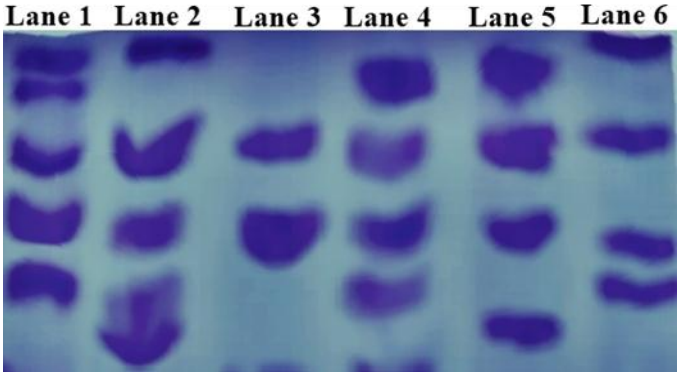
Fish, *Labeo rohita* of size 6±7 cm and 6.5±7.5 g weight were brought from a local fish farm Nandivelugu, Guntur district of Andhra Pradesh, India and acclimated at 28 ± 2^0C in the laboratory for 15 days. Such acclimated fish were exposed to sublethal ($1/10^{th}$ 96 hr LC_{50}10 µg L^{-1}) and carbosulfan ($1/10^{th}$ 96 hr LC50 i.e 0.12 mg/L) for 15 days. The vital tissues such as muscle, liver, gill and kidney of the fish were taken for the estimation of native PAGE of Lactate dehydrogenase (LDH).

Estimation of Lactate dehydrogenase

Native polyacrylamide gel electrophoresis (native PAGE) was performed to analyze lactate dehydrogenase (LDH) electrophoresis was carried out at 4^{o}C for 3 hr, applying a current of 100 mV. The procedure for LDH staining was based on method of Worthington with modifications [5]. The Staining solution for LDH comprised 0.1M Tris buffer(pH-8.4),1 mg ml^{-1} adenine dinucleotide(NAD^{+}),0.5 mg ml^{-1}, Nitro blue tetrazolium(NBT),0.1mg ml^{-1} Phenozine Metho Sulphate(PMS) and 0.05M Lithium lactate.

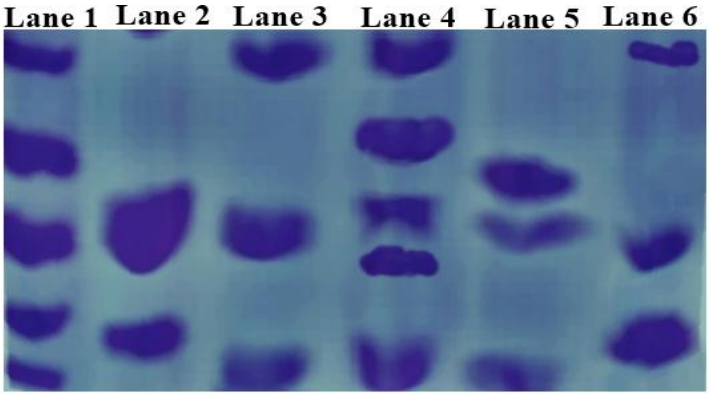
RESULTS AND DISCUSSION

The LDH profile in the muscle and liver tissues of control and both pesticides treated fish tissues was showed in Figure 1, amongst all lanes, lane 1 was found to be most intense. All bands in exposed tissues showed decrease in intensity when compared to control. The liver showed seven bands (lane 4-6) in control, profenofos and carbosulfan exposed.



Lane 1: control muscle
Lane 2: profenofos muscle
Lane 3: carbosulfan muscle
Lane 4: control liver
Lane 5: profenofos liver
Lane 6: carbosulfan liver

Fig.1: Changes in the activity of lactate dehydrogenase (LDH) in liver and muscle tissue of *L. rohita* on exposure to sublethal concentrations of profenofos and carbosulfan for 15 days



Lane 1: control gill
Lane 2: profenofos gill
Lane 3: carbosulfan gill
Lane 4: control kidney
Lane 5: profenofos kidney
Lane 6: carbosulfan kidney

Fig. 2: Changes in the activity of lactate dehydrogenase (LDH) in gill and kidney tissue of *L. rohita* on exposure to sublethal concentrations of profenofos and carbosulfan for 15 days

Amongst all lanes, lane 5 was the most intense. All bands in exposed tissues showed decrease in intensity when compared to control. The LDH profile in the gill and Kidney tissues of control and exposed tissues Figure. 2 and lanes 1-6.The gill showed five bands in control (lane 1), profenofos and carboslfan exposed (lane 2-3), amongst all lanes, lane 1 was the most intense. All bands in pesticides exposed tissues showed decrease in intensity when compared to control. The kidney showed five bands (lane 4) in control, profenofos and carbosulfan exposed, amongst all lanes, lane 4 was the most intense. All bands in exposed tissues showed decrease (lane 5-6) in intensity when compared to control. The difference of the distribution of LDH isoenzymes in tissues is known to reflect difference in their metabolic activity.

Jyothirmayee *et al*., [4] had done PAGE for endosulfan induced changes in LDH pattern in freshwater fish *Anabas testundineus* and *Clarias batrachus*. The protein subunits showed in intensity of all the fractions throughout the exposure periods, demonstrating an inhibitory effect of kidney and muscle LDH.

The decreasing inhibition of the LDH isoenzyme activity in muscle and in the bovine heart tissues of rabbits were studied by[6] after exposure to pentachlorophenol, an elevated level of LDH was observed in brain and liver tissues of lymphoma bearing mice by using native PAGE[7, 11]. Alterations in the LDH isoenzymes of tissues induced by toxic reflect a metabolic cellular dysfunction of these tissues[12,8,9]. Analysis of each tissue revealed characteristic changes in LDH isoenzyme patterns *in vivo* exposure to carbofuran[10].

CONCLUSION

In the present study, both toxicants treated samples showed a steady decreasing trend in intensity compared to control, demonstrating decreased activity of LDH on exposure to profenofos and carbosulfan. The decrease intensity was more in profenofos treated tissue than carbosulfan due to more pesticide stress.

REFERENCES

1. Velcheva I, TomovaE, Arnaudova D and Arnaudov A. Morphological investigation on gills and liver of freshwater fish from dam lake studen kladenets. Bulgarian journal of agricultural science. 2010; 16(3): 364-368.
2. Fujio Y and Kaneko S. Differentiation of LDH isoenzymes in fish. Tohoku Journal of Agricultural research. 1980; 31(1): 61-73.
3. Markert CL and Moller F. Multiple forms of enzyme tissues,ontogenetic and species specific patterns. Pro Nat Acad Sci. 1959; 45: 753-763.
4. Jyothirmayee S, Jane theophillus, Padma Balaravi, Narender Reddy T and Reddy PUM. Endosulfan induced changes in esterases of Anabas testudineus and Clarias Batrachus. Indian journal of comparative animal physiology. 2006; 24(1): 95-99.
5. Mishra, Rand Shukla SP. Immunochemical detection of Serum LDH1: An indigenous method in diagnosis of myocardial infraction.Current Science. 1999; 76(4): 587-590.
6. Young SJ, Dowman AA and Lowell DC. The detection of pentachlorophenol by its inhibitory effectiveness on lactate dehydrogenase of rabbit muscle and bovine heart. Pest Biochem Physiol. 1999; 64: 1-8.
7. Brij Bharti and Rajnikant Mishra. Lymphoma affects enzyme and protein profile of Non lymphatic tissue in mice. International Journal of Integrated Biology. 2008; 3(3): 175-181.
8. Ribeiro S, Guilhermino L, Sousa JP and Soares AMVM. Novel bioassay based on acetylcholinesterase and lactate dehydrogenase activities to evaluate the toxicity of chemicals to soil isopods. Ecotoxicol Environ Saf. 1999; 44: 287 – 293.
9. Tripathi G and Priyanka Verma. Endosulfan-mediated Biochemical Changes in the Freshwater Fish *Clarias batrachus*. Biomedical and environmental sciences. 2004; 17: 47-56.
10. Gupta RC Goad JT and Kadel WL. Invivo alterations in lactatedehydrogenase (LDH) and LDH isoenzymes patterns by acute carbofuran intoxication. Arch Environ Contam Toxicol. 1991; 21: 263-269.
11. Brij Bharti, and Rajnikant Mishra. Lymphoma affects enzyme and protein profile of Non lymphatic tissue in mice. International Journal of Integrated Biology. 2008; 3(3): 175-181.
12. Coquelle N, Fioravanti E, Weik M, Vellieux F and Madern D. Activity, stability and structural studies of Lactate dehydrogenases adapted to extreme thermal environments. J Mol Bio. 2007; 374(2): 547-562.