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A STUDY ON ENZYMES G-6-PASE AND ALP IN THE LIVER OF FISH *LABEO ROHITA* (HAMILTON) EXPOSED TO THE TOXICANTDICHLORVOS, AN ORGANO PHOSPHATE.

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ABSTRACT

Fishes are aquatic and poikilothermic animals. Hence, their existence and performance isdominated by the quality of their environment. Pollution of water bodies forces them toacclimatize to various factors thus imposing a considerable amount of stress on their lives.This ability to detect sudden changes in environment and monitoring short or long termchanges in water quality makes the fish efficient biomarkers. This study is aimed on the estimation of activity of Glucose 6 phosphatase (G-6-Pase) and alkaline phosphatase(ALP) (as biomarkers)in liver of Labeo rohita after the acute (4 days) and chronic exposure (30 days) of synthetic pesticide Dichlorvos. The 96h LC_{50} value of Dichlorvos determined by Finney's Probit Analysis Method (1971) was found to be 16.71 ppm.The results revealed that there was a significant increase in the G-6-Pase and ALP activity in the liver homogenate of treated fishes as compared to the control group. The elevated levels of G-6-Pase and ALP may be due to hepatotoxicity of liver cells. From the present study, it may be concluded that the analysis of enzyme activity of fishes can effectively be used as an indicator of fish health.

Key words: Dichlorvos, *Labeo rohita*, G-6-Pase and ALP activity, liver

INTRODUCTION

Among the pollutants, the pesticides have been identified and marked as one of major pollutants of the aquatic ecosystems with deleterious effects of either acute or chronic on the living inhabitants. They have been reported to produce a number of biochemical changes in fish, both at lethal and more often at sublethal levels. Fish species are sensitive to enzymatic as well as hormone disruption which is caused due to stress and pesticide effect. Chronic exposure to low levels, have more significant effect on fish populations than acute poisoning.

Enzymes are biochemical molecules that control metabolic processes of organisms, thus a slight variation in enzyme activities would affect the organism (Roy, S.S., 2002). Fish are excellent subjects for the study of various effects of contaminants present in water samples since they can metabolize, concentrate and store water borne pollutants. They are sensitive to contamination and the pollutants may damage some physiological and biochemical processes when they enter the organs of fishes (Tulasi *et al*., 1992). The changes in enzymatic system may alter the metabolic processes. More recently changes in enzymes concentrations are being employed in the evaluation of toxicological responses. Toxicologists have developed interest in studying the responses of individual enzymes or groups of enzymes to toxic insult. Several reports are available on the effect of insecticides on different aspects of metabolisms. Enzymes play significant role in food utilization and metabolism. Fish may be good indicators of contamination by pollutants because their biochemical responses are quite similar to those found in mammals (Banaee *et al*., 2008), According to Hassel (1990), biochemical changes occurs in fishes that are exposed to environmental contaminants, such changes which may include pesticides and their metabolites have necessitated a number of studies to determine their effects in aquatic environment on biochemical parameters in fish (Luskova *et al*., 2002).

Biochemical changes induced by pesticidal stress lead to metabolic disturbance, vital enzymes inhibition, retardation of growth and reduction of fecundity and longevity of organisms (Fatima, E., *et al*. 2006). Thus, by estimating the enzyme activities in an organism, we can easily identify disturbance in its metabolism. The use of biochemical measurements in organisms as indicators of pollution, give information about the adaptive or deleterious responses in organisms exposed to a certain amount of chemicals. Such analysis provides early warning signals before other toxicological points, including death are evident (Livingstone, 1998).

Glucose-6-phosphatase (G-6Pase) is an enzyme, which catalyzes the conversion of glucose-6-phosphate to glucose needed for vitellogenin synthesis. Glucose, which provides

energy for biosynthetic reactions including conversion of precursors to appropriate products, is phosphorylated by hexokinase to glucose-6-phosphate and eventually converted to pyruvate. Glucose-6-phosphatase (G6Pase), is an enzyme found mainly in the liver and the kidneys, plays the important role of providing glucose during starvation.Alkaline phosphatase (ALP) is a hydrolase enzyme responsible for removing phosphate groups from many types of molecules, such as, nucleotides, proteins and alkaloids.

Since the presence of the toxicant in water has been found to alter the physiology and biochemistry of fish there is, therefore, the need to examine the enzymatic changes associated with Dichlorvos-polluted environment in selected organ liver of *Labeo rohita* under laboratory conditions. Hence in the present study an attempt is made to study the effect of the toxicant, Dicholorvos an organophosphate on the enzymes Glucose 6 phosphatase (G-6-Pase) and Alkaline phosphatase (ALP) in the liver of fish *Labeo rohita* (Hamilton).

MATERIALS AND METHODS

Collection and acclimatization of experimental fish:

For the study of histopathological effects, live specimens of Healthy and active adult *Labeo rohita* were obtained from Patra and Bhadbhade fish farms barkhedi and bhadbhada Bhopal M.P respectively. They weighed 55±3gm and their length was in the range 14cm±1. They were brought to laboratory carefully in oxygen filled polythene bags in card board boxes to avoid any injury and disinfected by giving a bath for five minutes in $KMno_4$ solution. Thereafter, they were transferred to glass aquariums filled with dechlorinated water. The fishes were acclimated to the laboratory conditions for at least 20 days prior to the experiment. During acclimatization fishes were fed daily with commercial fish food which was given at morning hours. Water was replaced every 24h after feeding in order to maintain a healthy environment for the fish during acclimation and experimental period. This ensures sufficient oxygen supply for the fish and the environment is devoid of any accumulated metabolic wastes. Dead fishes when ever located were removed immediately to avoid fouling of the water.

Pesticide:

Dichlorvos manufactured by Sygneta India Ltd. 14, J. Tata road, Mumbai-400 020 was purchased from the local market and was used for evaluation of its toxicity to the fish.

Enzymological study:

In the present study, two sublethal concentrations ($1/10^{th}$ and 1/15th of LC_{50} values) of Dichlorvos were prepared and induced to *Labeo rohita* for 4 days (acute exposure) and 30 days (chronic exposure). Three groups were selected during the toxicity tests in which the group first was taken as the control group (no pesticide used) and the other two groups were given sub lethal concentrations of Dichlorvos. The group II was treated with $1/10^{th}$ concentration of 96hr LC_{50} value of Dichlorvos and the group III was treated with $1/15^{th}$ concentration of 96hr LC_{50} value of Dichlorvos. Six fishes were used in control as well as in experimental batches.

To study the activity of Glucose -6- phosphatase and Alkaline phosphatase homogenate of liver tissue from control and experimental fishes was prepared. For preparation of homogenate, fishes were dissected and the vital organ liver was removed, cleaned and homogenized in 0.25 M ice cold sucrose solution. Homogenates were then centrifuged for 15 minutes at 25,000 rpm in cooling centrifuging machine and clear supernatants were used as a source of enzyme. The enzyme extracts were kept at $0^{\circ}C$ until required.

For the Enzymological demonstration of G-6-Pase, method given by King (1965) was used and For the Enzymological demonstration of ALP, method given by Mod Kind and Kings (1954) was used.

RESULTS AND DISCUSSION

Enzymes are biochemical molecules that control metabolic processes of organisms, thus a slight variation in enzyme activities would affect the organism (Roy, S.S., 2002). Alteration in enzymes activities of the exposed fish is one of the major biomarker indicating the level of changes consequent of pollutants in the tissues, the organs and body fluid of the fish that can be recognized and associated with established health impairment process (Akinrotimi *et al.*, 2009). Moreover, Gabriel and Akinrotimi (2011) noted that biomarker can also be used to confirm and assess fish exposure to toxicants, providing a link between external exposure and internal structure and degree of responses to toxicant exposure observed between different individuals. The bio accumulation of certain persistent environmental contaminants in animal tissues may be considered to be biomarker exposure to these chemicals (WHO, 1993, Uner *et al.*, 2005).

GLUCOSE-6-PHOSPHATASE (G6Pase) IN LIVER OF *LABEO ROHITA*

Under acute exposure (4 days) to sublethal concentrations of dichlorvos 76% EC, the amount of Glucose 6 Phosphatase (G-6-Pase) was found to increase in the test tissue liver of *Labeo rohita* (**Table No. 1**). In the chronic exposure (30 days) to sublethal concentrations of dichlorvos, the amount of G-6-Pase also increased when compared to control (control): G-6-Pase (36.5 ± 0.04); (1.671 ppm): G-6-Pase (63.2 ± 0.31) and (1.44 ppm): G-6-Pase (54.3 ± 0.10) as illustrated in (**Table No. 2).**

ALKALINE PHOSPHATASE (ALP) IN LIVER OF *LABEO ROHITA*

Under acute exposure (4 days) to sublethal concentrations of dichlorvos 76% EC, the amount of Alkaline Phosphatase (ALP) was found to increase in the test tissue liver of *Labeo rohita* (**Table No. 3**). In the chronic exposure (30 days) to sublethal concentrations of dichlorvos, the amount of ALP also increased when compared to control (control): ALP (120.4 ± 0.04); (1.671 ppm): ALP (173.8 ± 0.17); (1.44 ppm): ALP (159.5 ± 0.41) as illustrated in (**Table No. 4)**

Table No. 1: Showing the activities of G-6-Pase in 4 days of Dichlorvos treated liver of *Labeo rohita.*

Dichlorvos Acute exposure (4 days)	**Glucose 6 phosphatase (G-6-Pase)**					
	Group – I (control)	**%age**	**Group – II (1.671ppm)**	**%age**	**Group– III (1.114 ppm)**	**%age**
	G-6-Pase (37.3±0.05)	100	G-6-Pase 46.1± 0.24*	123.59	G-6-Pase 40.4 ± 0.31*	108.31

Values are mean ± SD for 6 fishes in each group,*significant at $p<0.05$

Group II and III were compared with group I

Table No. 2: Showing the activities of G-6-Pase in 30 days of Dichlorvos treated liver of *Labeo rohita.*

Dichlorvos Chronic exposure (30 days)	**Glucose 6 phosphatase (G-6-Pase)**					
	Group – I (control)	**%age**	**Group – II (1.671ppm)**	**%age**	**Group– III (1.114 ppm)**	**%age**
	G-6-Pase (36.5± 0.04)	100	G-6-Pase 63.2 ± 0.31*	173.15	G-6-Pase 54.3±0.10*	148.76

Values are mean ± SD for 6 fishes in each group,*significant at $p<0.05$

Group II and III were compared with group I

Table No. 3: Showing the activities of ALP in 4 days of Dichlorvos treated liver of *Labeo rohita.*

Dichlorvos Acute exposure (4 days)	**Alkaline Phosphatase (ALP)**					
	Group – I (control)	**%age**	**Group – II (1.671ppm)**	**%age**	**Group – III (1.44 ppm)**	**%age**
	ALP(118.2 ± 0.06)	100	ALP (157.5 ± 0.19)*	133.24	ALP(133.7 ± 0.14)*	113.11

Values are mean ± SD for 6 fishes in each group,*significant at $p<0.05$

Group II and III were compared with group I

Table No. 4: Showing the activities of ALP in 30 days of Dichlorvos treated liver of *Labeo rohita.*

Dichlorvos Chronic exposure (30 days)	**Alkaline Phosphatase (ALP)**					
	Group – I (control)	**%age**	**Group – II (1.671ppm)**	**%age**	**Group– III (1.44ppm)**	**%age**
	ALP(120.4 ± 0.04)	100	ALP (173.8 ± 0.17)*	144.35	ALP(159.5 ± 0.41)*	132.47

Values are mean ± SD for 6 fishes in each group,*significant at $p<0.05$

Group II and III were compared with group I

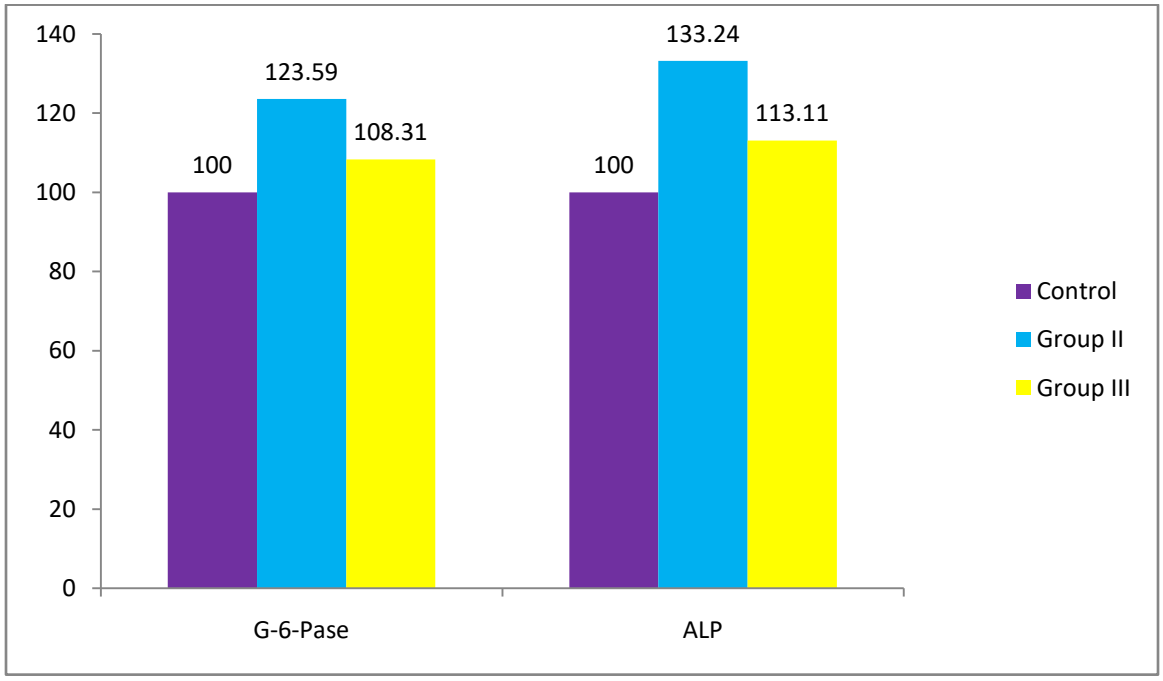

CHART NO. 1: Showing the comparison between the changes in G-6-Pase and ALP levels of liver in Dichlorvos treated fish, *Labeo rohita* during the acute exposure of 4 days.

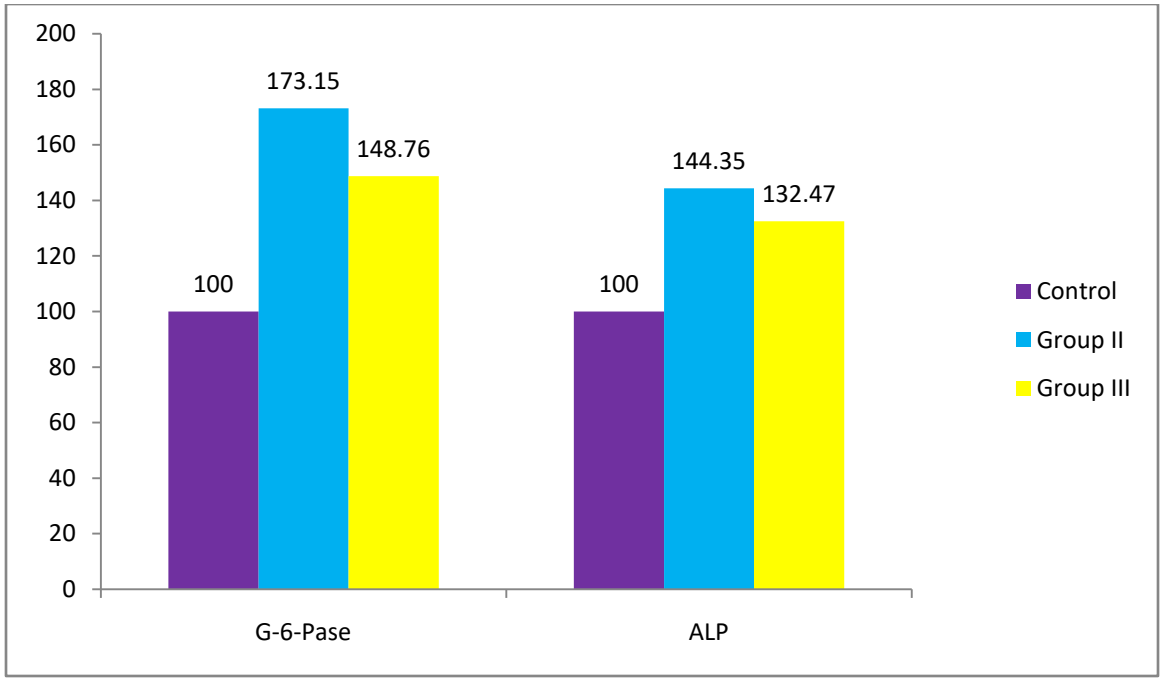

CHART NO. 2: Showing the comparison between the changes in G-6-Pase and ALP levels of liver in Dichlorvos treated fish, *Labeo rohita* during the chronic exposure of 30 days.

Sastry and Sharma (1980) observed the glucose-6-phosphatase activity in *Ophiocephalus* exposed to diazinon. Sastry and Sharma (1980) observed Glucose 6 phosphatase in *Nemachelius denisonii* exposed to phosphomidon. Elevation in glucose 6 phosphatase was observed in both the cases. Rashatwar and Ilyas (1984) studied the toxic effect of phosphomidon on glucose-6-phosphatase activity in the fish *Nemachelius.* Verma *et al*., (1984), identified the elevation of three phosphatases namely acid, alkaline and glucose-6-phosphatase in the serum of *Mystus vittatus* on exposure to the effects of pesticides thiotox, dichlorvos, carbofuran and their threecombinations. Elevation, in alkaline phosphatase was more than acid and glucose-6-phosphatases. Badre Alam Ansari and Kaushal Kumar (1987), drew inferences that the Zebra fish *Brachydanio rerio* (Cyprinidae) on malathion toxicity showed significant decrease in the activity of alkaline phosphatase in ovary tissues, the decrease in the alkaline phosphatase depended on both concentrations and time. Sastry *et al*., (1993) observed aldrin induced alterations in the activity of glucose-6-phosphatase in the freshwater teleost fish *Channa punctatus*. Srinivas (1993) studied the glucose-6-phosphatase in the fresh water fish *Clarias batrachus* which exposed to endosulfan. In all the cases there was an elevation in glucose-6-phosphatase activity. These results are in agreement with the present findings of the elevation in the G-6-Pase activity in the liver of studied fish *Labeo rohita* exposed to the pesticides neem-on

and dichlorvos.

The present findings are also in agreement of the following studies on different pesticides on fishes exposed to pesticides. David (1995) observed elevated levels of glucose-6-phosphatase activity in *Labeo rohita* exposed to fenvalerate. Medda *et al*.,(1995), subjected the fresh water carp fingerlings of *Labeo rohita* to rotenone sublethal concentrations. Sehgal *et al*., (2001) reported biochemical changes in the liver of the Indian freshwater murrel, *Channa punctatus* (Bloch) during estradiol-induced vitellogenin synthesis. Increased activity of G-6-Pase was observed in the exposed fishes. Das *et al*., (2006) reported metabolic elasticity and induction of heat shock protein 70 in *Labeo rohita* acclimated to three temperatures. Glucose 6 phosphatase (G6Pase, E.C. 3.1.3.9) of liver and kidney were significantly ($p<0.05$) different with increasing acclimation temperatures. Glucose-6-phosphatase activity showed a significant increase in the liver tissue of fish exposed to lethal and sublethal concentration of deltamethrin Rathnama*et al*., (2007).

Increase in ALP activity in liver and may be due to the uncoupling of phosphorylation by the insecticide. The changes in ALP depend on duration, concentration and the value of pesticide. Alkaline phosphatase (ALP) activities were increased in liver and muscle in three sublethal concentrations (0.0070%, 0.0140% and 0.0210%) after exposure to 24, 48, 72 and 96 hrs. and control group in freshwater fish; *Barilius burna* (Kamble *et al.,* 2011).

Jyothi and Narayan (1999) exposed the fresh water edible cat fish *Clarias batracus* (Linn.) to sublethal concentrations of two different groups of pesticides-carbaryl, a carbamate and phorate, an organophosphorus pesticide for 24, 74, 120 and 168 hours. They notified the increased level of alkaline phosphatase. Karthikeyan (2001), too noticed the increase in the activity of Alkaline phosphatase in serum followed by a decrease in the liver tissues of albino rats exposed to chronic administration of endosulfan. The effect of exposure to sublethal concentrations of the organophosphate pesticide, quinalphos (1.12, 0.22 mg) in muscle, brain, liver and kidney of the Indian major carp, *Labeorohita* was studied after 15, 30 and 45 days. The alkaline phosphatase was elevated (Das & Mukherjee 2000). These results are in agreement with the present findings of the elevation in the ALP activity in the liver of studied fish *Labeo rohita* exposed to the pesticides neem-on and dichlorvos. The present findings support these results. Ram and Singh (1988) found an elevation in ALP and ACP activity in the liver of carbofuran-treated *Channa punctatus*. There are reports indicating that increase in ALP could be a result of damage of liver cells and duct obstruction due to proliferation of its cells and/or related to the

progressive liver necrosis (Tietz, 1976). An increase in alkaline phosphatase activity was also noticed by Ruparelia et al. (1992) in Sarotherodon mossambica exposed to cadmium. Alkaline phosphatase splits various phosphate esters at an alkaline pH and mediates membrane transport. These results are in agreement with the present findings of the elevation in the ALP activity in the liver of studied fish *Labeo rohita* exposed to the pesticides neem-on and dichlorvos.

The present findings are also in agreement of the following studies on different pesticides on fishes exposed to pesticides. Nte *et al*., (2011) studied alterations in enzymes activities as a biomarker in black jaw tiliapia(*Sarotherodon melanotheron)*exposedto industrial effluents. ALP level was significantly increased during the exposure time. A comparative study on the effects of a pesticide (cypermethrin) and two metals (copper, lead) to serum biochemistry of Nile tilapia, *Oreochromis niloticus* was reported by Fırat *et al*., (2011). Effect of sublethal concentration of Dimecron on acid phosphatase (ACP) & alkaline Phosphatase (ALP) activities in liver, muscle & kidney of a freshwater fish; *Barilius burna* was investigated by Kamble *et al*., (2011). Assessment of anthracene on hepatic and antioxidant enzyme activities in *Labeo rohita* (Hamilton, 1822) was reported by Vasanth *et al*., (2012).

These results indicate that concentrations of Dichlorvos have a toxic effect on fishes and changes the phosphatases activities which in turn will affect the overall health of the fish. It is suggested that the use of pesticide in aquatic ecosystem should be minimized to prevent the effects. Therefore this pesticide should be used with great caution and in a sustainable way so that it may not be hazardous to aquatic biota and human beings.

Conclusion

The results of the present study clearly indicate the toxic nature of the insecticide dichlorvos on the biochemical constituents of the studied fish *Labeo rohita*. It is therefore necessary to focus attention on changes in biochemical composition of aquatic organisms, which are under pollutant threat.In addition, potential risk from Dichlorvos metabolites should be investigated to get a more complete picture in terms of toxicity.

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