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RESEARCH ARTICLE

ENZYMOLOGICAL CHANGES INDUCED BY QUINALPHOS IN THE TISSUES OF FRESH WATER FISH, *LABEO ROHITA*

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ABSTRACT

Enzymes are biological catalysts produced by living cells they catalyze metabolic reactions. They are soluble and colloidal substances characterized by great activity, specificity and susceptibility to the influence of environmental changes. Enzymes are the most important tools of the living cells. Cells cannot be without enzymes. They function as catalysts in a wide variety of biological reactions. Activity levels of insecticide, Quinalphos were studied in the different tissues of fresh water fish, *Labeo rohita* exposed to sublethal concentration (6.06 ppm) during different exposure periods of 1 day, 2 days, 3 days, 10 days and 20 days. The levels of enzymes like GOT, GPT and LDH were noticed in gill, muscle, liver and kidney, tissues with timely course of study. The fish treated with Quinalphos showed greater inhibition of enzymes during the study period.

INTRODUCTION

The aquatic environment where fish and other aquatic organisms live is subjected to different type of pollutants which enter water bodies through industrial, domestic and agricultural discharge systems there by introducing stress to living creatures. Stress is general and non specific response to any factor disturbing homeostasis. Stress in fish may be induced by various abiotic environmental factors like changes in water temperature, pH, oxygen concentration and pollution. Changes in environmental quality can therefore be a major determinant of year class strength and eventually the long term dynamics of many fish population. The traditional use of pesticides in agriculture to control the insect pests for obtaining better yield of crops has resulted adversely on aquatic crop of fish farming. This is because of the fact that these chemicals ultimately find their way from the agricultural fields to our freshwater bodies where they affect the fish as well as other aquatic life. It is also known that a good number of newer pesticides are introduced and the farmers are advised to use them for their better yield purpose .It is therefore, necessary to test the toxicity of such pesticides particularly to fish. The elevated lactated dehydrogenase (LDH) activity is marker for tissue damage in fish hypoxic a conditions and muscular harm and serve as a good diagnostic tool in toxicology. Glutamate dehydrogenase (GDH), a mitochondrial enzyme, catalysis the oxidative deamination of glutamate, providing ketoglutarate to the kreb's cycle (Reddy and Venugopal, 1990). This enzyme having several metabolic function with great physiological significance.

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It is closely associated with the detoxification mechanisms of tissues. GDH in extra hepatic tissues could be utilized for channeling of ammonia released during proteolysis for its detoxification in to urea in the liver. Hence, the activities of GDH are considered as sensitive indicators of stress. The persistence of toxic chemicals in aquatic environment becomes dangerous for the survival of fish and their food organisms. Therefore it is essential to study the toxic effects of pesticides on living organisms.

MATERIALS AND METHODS

The fresh water fish, *Labeo rohita* (body length 5-7 cm, body weight 5-6 gm) were collected from Aliyar Dam and acclimatized to laboratory condition for 2 weeks in a large cement tanks (6 x 4 x 3) at (24 ± 3°C). The fishes were fed regularly with conventional diet rice bran and oil cake 1: 1 ratio feeding was stopped one day prior to the start of the experiment . Technical grade of Quinalphos, a insecticide was used in the investigation. Bathes of 10 healthy fishes were exposed to different concentration of the insecticide. LC_{50} value for 72 hrs was calculated by using probity analysis (Finney, 1971). Five groups of fishes were exposed to 6.06ppm (sublethal concentration) of 72 hrs LC_{50} valued concentration of the Quinalphos for 1, 2, 3, 10 and 20 days respectively. Another group was maintained as control at the end of each exposure period. Fishes were scarificed and tissues such as liver, kidney, muscle and gill were dissected and removed. The tissues were homogenized with 80% methanol centrifuged at 3500 rpm for 15 minutes and the clear supernatant was used for analysis of different parameters. The results were expressed as mg/g wet weight of the tissue. Different enzymological parameters like ACP and ALP were analysed.

RESULTS AND DISCUSSION

Changes in the GOT activity in the gill, muscle, liver and kidney of the fish, *Labeo rohita* exposed to Quinalphos were presented in the Tables 1, 2 and 3. During the above treatment kidney and muscle showed a maximum percentage increase (98.44 and 97.18) 3 days exposure time followed by liver (87.17) during 3 days and gill (86.92) during 20 days exposure respectively.

The GPT activity in the organs of fish, *Labeo rohita* when treated with Quinalphos in short term and long term exposure periods. During Quinalphos treatment the enzyme activity was increased in muscle, kidney, liver and gill. The fish showing a percentage increase of muscle and gill (98.26 and 90.80), during 20 days. The fish showing a percentage increase of 87.62 and 97.31 during the periods of 3 days in liver and kidney respectively.

Table 1. Glutamate oxaloacetate transaminase activity (IU/L) in the tissues of *Labeo rohita* on exposure to insecticide, Quinalphos

Name of the Tissues		Exposure Periods				
		1 day	2 days	3 days	10 days	20 days
Liver	Control	57.61 ± 0.16	57.61 ± 0.16	57.61 ± 0.16	57.61 ± 0.16	57.61 ± 0.16
	Experimental	16.99 ± 0.05***	9.01± 0.02***	7.39± 0.08***	11.28±0.06***	9.21± 0.00***
	't' value	7.56	1.80	2.64	2.64	1.21
	% change	+70.51	+84.36	+87.17	+80.42	+84.02
Gill	Control	107.41 0.06	107.41± 0.06	107.41 ± 0.06	107.41 ± 0.06	107.41 ± 0.06
	Experimental	56.95 ± 0.02***	39.19±0.05***	24.62±0.03***	21.20±0.47***	14.05±0.06***
	't' value	1.33	1.19	2.54	1.19	9.70
	% change	+46.98	+63.51	+77.08	+80.26	+86.92
Kidney	Control	41.16 ± 0.03	41.16 ± 0.03	41.16 ± 0.03	41.16 ± 0.03	41.16 ± 0.03
	Experimental	4.66 ± 0.02***	1.39 ± 0.00***	0.644±0.01***	4.86 ± 0.06***	3.20 ± 0.01***
	't' value	6.94	6.54	6.80	9.70	1.98
	% change	+88.68	+96.64	+98.44	+88.19	+92.22
Muscle	Control	71.64 ± 0.06	71.64 ± 0.06	71.64 ± 0.06	71.64 ± 0.06	71.64 ± 0.06
	Experimental	6.01 ± 0.02***	4.64 ± 0.03***	2.02 ± 0.05***	3.21±0.06***	2.07±0.01***
	't' value	1.63	1.38	1.01	1.16	6.63
	% change	+91.61	+93.52	+97.18	+95.52	+97.12

Results are mean (±SD) of 6 observations; % = percent increase / decrease over control;
* = Significant at 0.5 level; *** = Significant at 0.001 level.

Table 2. Glutamate pyruvate transaminase activity (IU/L) in the tissues of *Labeo rohita* on exposure to insecticide, Quinalphos

Name of the Tissues		Exposure Periods				
		1 day	2 days	days	10 days	20 days
Liver	Control	49.25 ± 0.01	49.25 ± 0.01	49.25 ± 0.01	49.25 ± 0.01	49.25 ± 0.01
	Experimental	18.85 ± 0.05***	10.33±0.06***	6.1± 0.32***	9.61±0.08***	6.59± 0.02***
	't' value	4.99	6.91	2.91	3.97	3.32
	% change	+61.73	+79.03	+87.62	+80.49	+86.62
Gill	Control	87.67± 0.05	87.67± 0.05	87.67 ± 0.05	87.67 ± 0.05	87.67 ± 0.05
	Experimental	41.35 ± 0.02***	26.54±0.03***	17.38±0.05***	11.00±0.80***	8.07±0.03***
	't' value	2.64	2.88	9.40	2.93	3.47
	% change	+52.83	+69.73	+80.18	+87.45	+90.80
Kidney	Control	39.61 ± 0.08	39.61 ± 0.08	39.61 ± 0.08	39.61 ± 0.08	39.61 ± 0.08
	Experimental	7.48 ± 0.03***	2.67 ± 0.02***	1.065±0.01***	6.62 ± 0.08***	2.05 ± 0.01***
	't' value	3.08	1.62	7.47	3.99	9.20
	% change	+81.12	+93.26	+97.31	+83.29	+94.82
Muscle	Control	67.10 ± 0.05	67.10 ± 0.05	67.10 ± 0.05	67.10 ± 0.05	67.10 ± 0.05
	Experimental	5.34 ± 0.02***	3.21 ± 0.05***	1.94 ± 0.06***	2.68±0.03***	1.25±0.08***
	't' value	1.50	1.16	1.01	1.09	9.29
	% change	+92.55	+95.52	+97.29	+96.26	+98.26

Results are mean (±SD) of 6 observations; % = percent increase / decrease over control;
* = Significant at 0.5 level; *** = Significant at 0.001 level.

Table 3. Lactate dehydrogenase activity (IU/L) in the tissues of *Labeo rohita* on exposure to insecticide, Quinalphos

Name of the Tissues		Exposure Periods				
		1 day	2 days	3 days	10 days	20 days
Liver	Control	4.31 ± 0.02	4.31 ± 0.02	4.31 ± 0.02	4.31 ± 0.02	4.31 ± 0.02
	Experimental	3.11 ± 0.06***	2.17±0.047***	1.99± 0.00***	1.51±0.05***	0.91± 0.02***
	't' value	8.35	8.22	1.37	1.33	2.52
	% change	+27.88	+49.68	+53.85	+64.98	+78.90
Gill	Control	7.95 ± 0.02	7.95± 0.02	7.95 ± 0.02	7.95 ± 0.02	7.95 ± 0.02
	Experimental	4.31 ± 0.03***	3.94±0.05***	1.99±0.06***	2.55±0.08***	1.21±0.02***
	't' value	1.18	5.43	2.28	5.02	8.53
	% change	+45.80	+50.50	+74.97	+67.93	+84.78
Kidney	Control	6.032 ± 0.01	6.032 ± 0.01	6.032 ± 0.01	6.032 ± 0.01	6.032 ± 0.01
	Experimental	1.49 ± 0.03***	0.98 ± 0.06***	0.01±0.01***	1.86 ± 0.08***	0.41 ± 0.02***
	't' value	2.01	8.58	3.25	3.98	3.65
	% change	+75.30	+83.75	+99.78	+69.16	+93.20
Muscle	Control	5.64 ± 0.02	5.64 ± 0.02	5.64 ± 0.02	5.64 ± 0.02	5.64 ± 0.02
	Experimental	2.01 ± 0.02***	1.87 ± 0.03***	0.94 ± 0.05***	2.94±0.08***	0.97±0.06***
	't' value	1.67	1.65	1.51	1.82	1.52
	% change	+64.36	+66.84	+83.33	+47.87	+82.80

Results are mean (±SD) of 6 observations; % = percent increase / decrease over control;
* = Significant at 0.5 level; *** = Significant at 0.001 level.

During short term exposure period of *Labeo rohita* to Quinalphos, the LDH activity was increased in kidney and muscle, showing a percent increase of 99.78 and 83.33 respectively.

In liver (78.90) gill (84.78) showed maximum increase during the exposure period of 20 days. The values are significant at 0.001 level. Liver is the metabolic centre for detoxification of chemicals.

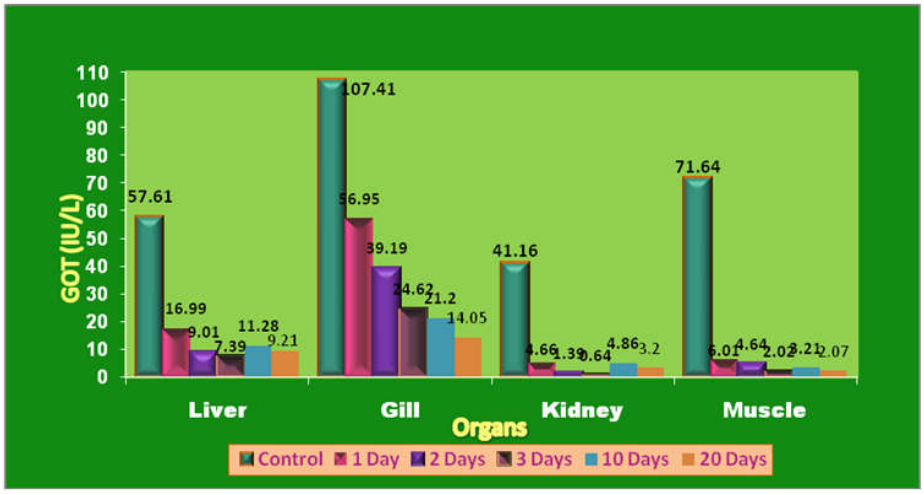

Figure 1. Level of GOT (IU/L) in the tissues of *Labeo rohtia* exposed to insecticide Quinolphos in different periods

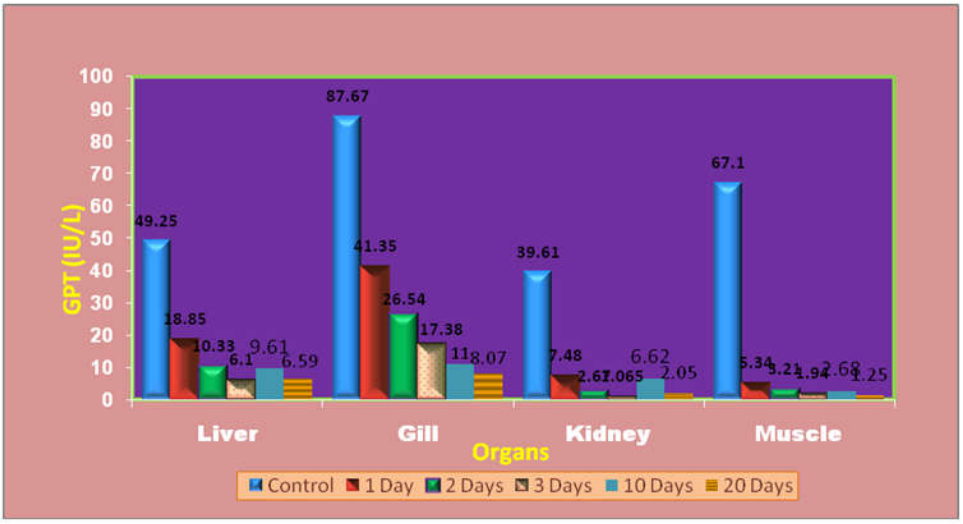

Figure 2. Level of GPT (IU/L) in the tissues of *Labeo rohtia* exposed to insecticide Quinolphos in different periods

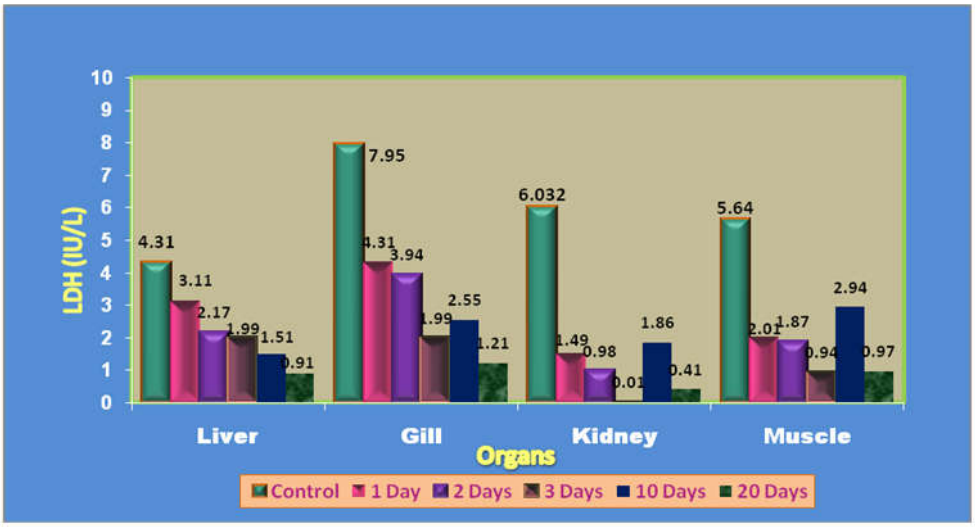

Figure 3. Level of LDH (IU/L) in the tissues of *Labeo rohtia* exposed to insecticide Quinolphos in different periods

Liver damage was conformed by changes in the activities of GOT and GPT activities. Increase the concentration of pesticide the hepatic tissue damaged much more. This is due to increased activities of GOT and GPT Agrahari *et al.* (2007). In the present study the percentage of GOT in different tissues (Gill, muscle, kidney and liver) during different exposure periods (Both short term and long term) are low. This may be increase in metabolic activity sites and tissue damage. Similar observations were made by Bhatnagar and Tyagi, (1995). The depletion in the activity of GPT indicates disruption of link between carbohydrates and protein metabolism providing some of keto acids for Kreb's cycle and gluconeogenesis. The pesticide intoxicants strongly depressed GPT activites as a consequences of serious cellar structure damage.

GOT and GPT are important in the diagnosis of heart and liver damage (Dere and Polat, 2001). The observed changes could be due to generalized organ system failure of fish due to the effect of Quinalphos. Latic dehydrogenase (LDH) forms the centre fore a delicately balanced equilibrium between catabolism and anabolism of carbohydrates LDH mediates interconversion of lactate to pyuvate depending on the availability of NAD, Co- enzyme. The decrease in lactate activity with a consequent increase in the levels of lactic acid suggests the predominance of anaerobic segment, glycolysis. LDH increased permeability of cell and necrosis are usually characterized by rise in LDH activity. LDH level which indicate the energy demands are met by anaerobic respiration through increase in LDH activities in stressed animals. They suggested that the stressed animals are meeting its energy requirement through anaerobic oxidation (Das and Mukherjee, 2000).

The increase in LDH level indicated metabolic changes, i.e. the glycogen catabolism and glucose shift towards the formation of lactate in stressed fish, primarily the muscle tissue. The increase of LDH activity is considered as an indicator of liver damage (Chen *et al*., 2000) observed a significant rise in serum LDH activity after liver infection. They referred the augmentation due to muscle LDH release into the blood stream. Sharma (1999) reported that significant decrease in the activity of liver LDH suggests that anaerobic metabolism was favored over aerobic oxidation of glucose through Kreb's cycle in order to mitigate the energy crisis for survival. A reduction in the concentration of LDH in the plasma of the experimental fish infers a decrease in the glycolytic process due to liver metabolic rate, a shift towards anaerobic respiration (Tiwari and Singh, 2004), possibly due to a hypoxic thermal environment. In the present study the toxicities of organophosphorous insecticide, Quinalphos cause on many adverse effect on many tissues such gill, muscle, liver and kidney, of the fresh water fish, *Labeo rohita*.

The organophosphates can induce oxidative stress by generating free radicals and altering antioxidant level of the free radical scavenging enzyme activity.

Conclusion

It is concluded that the insecticide induced alterations in the activities of the enzymes like GOT, GPT and LDH and these enzymes may be used as logical candidates to monitor the toxic level of insecticide and its impact on aquatic organisms. This insecticides are commonly used in agriculture field for achieving better quality products are toxic substances and lead to generation of reactive oxygen species.

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