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Evaluation of Liver Dysfunction and Metabolic Enzyme Modulation in Freshwater Fish Exposed to Lambda-Cyhalothrin

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ABSTRACT

The metabolic processes and in detoxification of xenobiotics liver plays an important role. This study examined the toxicological effects of lambda cyhalothrin on liver function of freshwater catfish focusing on liver marker enzymes, gluconeogenic enzymes and cytochrome p450 protein. Specific activity of Aspartate Amino Transferase (AST) and Alanine Amino Transferase (ALT), specific activity of Alkaline Phosphatase (ALP) and Acid Phosphatase (ACP), Specific activity of Alkaline Phosphatase (ALP) and Acid Phosphatase (ACP), Specific activity of Phosphoenol Pyruvate Carboxykinase (PEPCK) and the specific activity of pyruvate carboxylase were tested. The hepatic activity of the liver marker enzymes Alanine Amino Transferase (ALT) and Aspartate Amino Transferase (AST) varied significantly ($P < 0.05$) over the period of exposure. Increased phosphatase activity was observed with 45.21% and 7.07% in fish exposed to higher and lower sub-lethal doses, respectively, despite an overall significant drop in acid phosphatase (ACP) and alkaline phosphatase (ALP) activities ($P < 0.01$). Although it decreased at the end of the exposure, the gluconeogenic enzyme Phosphoenol Pyruvate Carboxykinase (PEPCK) was still considerably higher ($P < 0.05$) than the control levels. Additionally, even on day 45, gamma-glutamyl transferase (GGT) activity remained elevated ($P < 0.05$). The amount of cytochrome P450 (CYP450) changed dramatically ($P < 0.05$) over the course of exposure in both experimental indicating disrupted detoxification pathways.

INTRODUCTION

The liver is an important organ involved in metabolic processes and in detoxification of xenobiotics. In certain situations, the toxic substance accumulation may exceed toxic levels and cause pathological alterations (Ferguson, 1989; Braunbeck et al., 1990). The occurrence of acid phosphatase and alkaline phosphatase enzymes in tissues is believed to be associated with the transport of metabolites and metabolism of phospholipids, proteins and nucleotides (Srivastava et al., 1995).

Liver is a large and metabolically most complex organ in the body. It is involved in the metabolism of nutrients as well as many drugs and toxicants. Liver injury is dependent not only on the particular agent but also its mechanism of action and length of exposure (Jacobson Kram and Keller, 2001). Toxicants are bound to significantly damage certain physiological and biochemical processes when they enter the organs of fishes (Murty, 1986; Teh et al., 1997). In toxicological studies of acute exposure, changes in the activity of enzymes often directly reflect cell damage in specific organs (Casillas et al., 1983). Liver is a major metabolic and detoxification site in animals. Any toxic substance when enters the body has to pass through the liver and may thus bring about various pathological changes in the hepatic cells which is evident from the increased levels of certain biomarker enzymes which are being used predominantly in toxicological studies, to assess the extent of damage brought about by the toxicant.

The concentrations of ACP and ALP in the haemolymph, gills and muscle tissue of prawn, *Macrobrachium malcolmsonii* were reported to be significantly declined on exposure to endosulfan (Bhavan and Geraldine, 2004). Jana et al. (1985) also observed a significant decline in the ACP and ALP levels in the freshwater fish, *Clarias batrachus* on exposure to mercury. Studies on the effect of a sub-lethal concentration of carbofuran on air breathing catfish, *Clarias batrachus* also showed a significant decline in the acid and alkaline phosphatase enzyme levels in the vital tissues of the fish (Mukhopadhyay et al., 1982).

The decrease in the tissue ACP and ALP levels can be correlated with their subsequent increase in the serum due to the leakage of these enzymes from the tissues into the serum. Another important group of liver biomarker enzymes are the transaminases which play an important role in the utilization of protein and carbohydrates. It has been suggested that stress induces the transamination pathway has been reported in the earlier findings of Malla Reddy and Bashamohideen (1995). Fishes are known to respond to stressful conditions by gearing up their metabolic activity by elevation of transaminases. Sharma (1999) also reported a marked increase in the activities of transaminases in the serum of *Clarias batrachus* treated with carbamyl, a carbamate pesticide for a period of 15 days, the

magnitude of the effect being dependent on the pesticide concentration and duration of exposure.

Gamma glutamyl transpeptidase (GGT) is a liver enzyme involved in the transfer of amino acids across the cellular membranes and plays a role in glutathione metabolism. Gamma-Glutamyl transpeptidase (GGT) is the only enzyme known that can cleave the gamma-peptide bond between glutamate and cysteine in glutathione, and is therefore a key enzyme in glutathione degradation. Exogenous chemicals introduced into organism may undergo chemical metabolic transformation termed transformation, process biotransformation. Transformation is accomplished by enzymes and results in either the alteration of the foreign chemical or the formation of conjugated products. The new product may be toxic than the parent compound. The activity of fish monooxygenases has been used as a monitoring tool to evaluate contamination by cytochrome P450 inducing agents (Figueiredo-Fernandes et al., 2006). Cytochrome P450 is an important family of oxidation enzymes that are involved in the oxidation, reduction and hydrolysis reactions of the metabolism of a majority of xenobiotics. This is an attempt to study the effect of lambda cyhalothrin on liver marker enzymes, gluconeogenic enzymes and cytochrome p450 protein content.

MATERIALS AND METHODS:

Specific activity of Aspartate Amino Transferase (AST) and Alanine Amino Transferase (ALT):

The tissue and serum aspartate aminotransferase activity; the tissue and serum alanine aminotransferase activity was assayed following the method of Reitman and Frankel (1970). The specific activity of AST was expressed as µmoles of pyruvate liberated/hour/mg protein and the specific activity of ALT was expressed as moles of pyruvate formed/hour/mg protein.

Specific activity of Alkaline Phosphatase (ALP) and Acid Phosphatase (ACP):

Alkaline phosphatase (ALP) and Acid Phosphatase (ACP) activity in the tissue and serum was assayed by the method of Bergmeyer (1963). The specific enzyme activity was determined as millimoles of phenol liberated/hour/mg protein.

Specific activity of Phosphorylase:

The specific activity of phosphorylase was assayed following the procedure of Wootton (1964). The inorganic phosphorous liberated was assayed according to the method of Fiske and Subbarow (1925). The specific enzyme activity was expressed in terms of umol of inorganic phosphorous formed/hr/mg protein.

Activity of Gamma-Glutamyl Transferase (GGT):

The serum gamma-glutamyl transferase activity was assayed following the method of Szasz, 1974. The GGT content was expressed as IU/l.

Specific activity of Phosphoenol Pyruvate Carboxykinase (PEPCK):

The specific activity of phosphoenol pyruvate carboxykinase was assayed in the hepatic tissue following the procedure of Mommsen et al. (1985). The specific activity of phosphoenol pyruvate carboxykinase was expressed as units/g wet wt.

Specific activity of Pyruvate Carboxylase (PC):

The specific activity of pyruvate carboxylase was assayed following the procedure of Foster and Moon (1986). The specific activity of pyruvate carboxylase was expressed as units/g wet wt.

Assay of liver microsomal cytochrome P450:

A. Isolation of microsomes: Microsomes were isolated from the hepatic tissue following the procedure of Besselink et al. (1996).

B. Assay of cytochrome P450: Total microsomal Cytochrome P450 content was determined by the method of Omura and Sato (1964).

RESULTS:

The alterations in the biomarker enzyme activity, gluconeogenic enzyme activity cytochrome P450 content of the hepatic tissue of the pyrethroid-exposed fishes are tabulated (Table 1a & 1b).

The fishes exposed to both the sub-lethal concentrations of the pyrethroid showed an overall significant increase ($P < 0.01$) in the activity of aspartate amino transaminase (AST) and alanine amino transaminase (ALT) of the hepatic tissue. A comparison of the ALT and AST activity in the hepatic tissue of pyrethroid-exposed fishes at different durations of exposure also showed significant variations ($P < 0.05$) in the levels of both these enzymes in both groups of pyrethroid-exposed fishes.

An overall significant decline ($P < 0.01$) was witnessed in the hepatic activity of acid phosphatase (ACP) and alkaline phosphatase (ALP) in fishes exposed to the two different sub-lethal concentrations of lambda cyhalothrin. A gradual and significant decline ($P < 0.05$) was witnessed in the acid phosphatase activity of both groups of pyrethroid-exposed fishes in comparison with the control group. A similar trend was witnessed in the activity of alkaline phosphatase enzyme in the fishes exposed to the two different sub-lethal concentrations of the pyrethroid.

The phosphorylase enzyme activity of the hepatic tissue of the pyrethroid-exposed fishes of both groups showed a significant elevation ($P < 0.05$) in comparison to the enzyme activity in the control group of fishes. However, in both groups, a significant elevation was witnessed in the enzyme activity only on the 30^{th} and 45^{th} day of exposure to the pyrethroid, while no significant change was witnessed on the 15^{th} day. The phosphatase enzyme activity showed an overall elevation of 45.21% and 7.07% in fishes exposed to the higher and lower sub-lethal concentration of the pyrethroid respectively.

The activity levels of the enzyme, phosphoenolpyruvate carboxykinase (PEPCK) showed a significant elevation ($P < 0.05$) on the 15^{th} and 30^{th} day of exposure to the pyrethroid in both groups of experimental fishes when compared to the control group of fishes. However, the initial increase in enzyme activity was followed by a decline with an increase in the duration of exposure. Though the PEPCK activity was found to decline towards the end of the exposure period, the values still remained significantly elevated ($p < 0.05$) beyond the control levels.

A similar trend was also witnessed in the changes in the activity of pyruvate carboxylase of the hepatic tissue in both groups of pyrethroid-exposed fishes. The 30^{th} day showed a significant elevation ($P < 0.05$) of 29.87% and 29.37% in the enzyme activity of the fishes exposed to the higher and lower sub-lethal concentrations of the pyrethroid respectively when compared to the control group of fishes. However, by the 45^{th} day of exposure, though the pyruvate carboxylase activity showed a decline, the enzyme activity was still found to be elevated by 22.40% and 14.24% over the control. However, both groups of experimental fishes showed an overall significant change ($P < 0.01$) in the enzyme activity.

The activity of gamma glutamyl transferase (GGT) was also found to be significantly elevated ($P < 0.05$) over the control levels on the 30^{th} day of exposure in fishes of both experimental groups. The fishes exposed to higher sub-lethal concentration of the pyrethroid showed an elevation of 154.81% in the GGT activity, whereas the fishes exposed to the lower sub-lethal concentration showed an elevation of 153.63%. The increase in the GGT activity was followed by a decline as witnessed on the 45^{th} day of exposure of the fishes to the pyrethroid. However, though a decline was witnessed in the enzyme activity towards the end of the exposure period, the GGT activity was found to be significantly elevated ($P < 0.05$) beyond the control levels even on the 45^{th} day of exposure.

The CYP450 protein content assayed in the hepatic tissue of the fishes after exposure to the two different sub-lethal concentrations of the pyrethroid showed a significant enhancement up to the 30^{th} day of exposure to the pyrethroid. This was followed by a significant decline ($P < 0.05$) in the ($P < 0.05$) CYP450 content and an overall decline of 83.18% and 23.53% was witnessed in the CYP450 content after 45 days of exposure to the pyrethroid. A comparison of the CYP450 content of the hepatic tissue at different durations of exposure to the pyrethroid showed significant variation ($P < 0.05$) in both experimental groups.

DISCUSSION

Liver is a large and metabolically most complex organ in the body. It is involved in the metabolism of nutrients as well as many drugs and toxicants. Liver injury is dependent not only on the particular agent but also its mechanism of action and length of exposure (Jacobson Kram and Keller, 2001). Stress is generally known to elevate the aminotransferase activity (Natarajan,

1985). The increase in the activity of ALT and AST enzymes during toxicant stress indicates the severity of hepatic damage (Ginsberg, 1970).

The present study reported a significant increase in the hepatic ALT and AST levels on exposure to both the sub-lethal concentrations of lambda cyhalothrin probably due to increase the metabolic rate of the animal under conditions of toxicant induced stress. The present findings gain the support of the previous findings of Singh and Gupta (2005) in *Heteropneustes fossilis* exposed to lithium, Shobha Rani et al. (2001) in fresh water teleost, *Tilapia mossambica* exposed to arsenite and in *Channa punctatus* during biochemical stress of *Nerium indicum* leaf extract by Tiwari and Singh (2004).

The rise in enhanced protein catabolism and probable hepatocellular damage in the activities of transaminases due to pesticide intoxication suggests organism (Sharma, 1999). However, contrary to the present findings, Humtsoe et al. (2007) reported a significant decline in the GOT, GPT levels of juvenile *Labeo rohita* exposed to arsenic.

ACP acts as a good indicator of environmental stress condition in the biological system (Gill and Jaishree Hema, 1990). Alkaline phosphatase on the other hand splits various phosphorous esters at alkaline pH, mediates membrane transport and is involved in glycogen metabolism. ALP is involved in the permeability processes and forms a part of the enzyme system Involved in the active synthesis of protein from nucleic acid complex (Jana et al., 1985).

In the present study a significant decline was witnessed in the ALP and ACP levels of the liver tissue. The inflammatory and necrotic changes in tissues due to the toxic exposure might have resulted in the cellular and lysosomal membrane rupture leading to decreased levels of ALP and ACP in the hepatic tissue (Bhavan and Geraldine, 2004).

The decreased activity of ACP and ALP indicate disturbance in structural integrity of cellular organelles like endoplasmic reticulum and membrane transport system (Humtsoe et al., 2007), Such damage to cell organelles has been reported in various earlier studies (Roy, 2002). The variations in the hepatic levels of phosphatases in the present study could also be attributed to similar disturbance in the structural and functional integrity of the cellular organelles of the hepatic tissue of *Clarias batrachus* exposed to sub-lethal concentrations of lambda cyhalothrin

The results of the present study are in correlation with the previous findings of *Macrobrachium malcolmsonii* exposed to endosulfan (Bhavan and Geraldine, 2004) Cyprinus carpio fingerlings exposed to lead (Pugazhendy et al., 2007). Significant attenuation in the level of phosphatases was also reported in *Labeo rohita* after 7 days of cypermethrin exposure (Philip and Anuradha, 1996).

In the present study, exposure of *Clarias batrachus* to the sub-lethal concentration of the pyrethroid led to a significant increase in the phosphorylase activity in the hepatic tissue which serves as a main portal for glycogenolysis.

There is ample evidence that cortisol increases all key gluconeogenic enzymes including PEPCK in fish liver (Mommsen et al., 1999). Thus the increased levels of cortisol in the serum could have also brought about the increase in the liver PEPCK activity in the present study. Levesque et al. (2002) reported a significant elevation in the PEPCK activity of the liver tissue in yellow perch, *Perca flavescens* chronically subjected to heavy metal pollution. In the present study, the initial increase in the activity of PEPCK was followed by a significant decline in the enzyme activity with the increase in the duration of exposure to the toxicant which may probably be due to direct impact of the toxic compound on the enzyme with the increase in the duration of exposure, Inhibiting its action (Viluksela et al., 1999). Goswami et al. (2004) reported a significant decline in the PEPCK activity of the liver of *Clarias batrachus* subjected to osmotic stress.

In the present study, an increased activity of pyruvate carboxylase was reported in the hepatic tissue from the 15th day of exposure which is in line the exposure period; a decline was witnessed in the enzyme activity. Rathman et al. (2003) showed that dietary carbamazepine administration decreased liver pyruvate carboxylase activity in rats.

In the present study, a significant elevation was reported in the activity levels of gamma glutamyl transpeptidase (GGT) in the hepatic tissue of the experimental fishes exposed to both the sub-lethal concentrations of lambda cyhalothrin during the initial periods of exposure which was followed by a significant decline. Favari et al. (2002) reported an increase in the GGT activity in the fish of Ignacio Ramirez reservoir (Mexico) in response to the environmental stress caused by the elevated biomagnification of organochloride and organophosphate pesticides. The increased utilization of amino acids as a source of energy to sustain the metabolic pathway under conditions of stress could have led to the enhanced levels of GGT in the hepatic tissue of fishes exposed to sub-lethal concentration of the pyrethroid in the present study.

In the present study, the hepatic microsomal Content of CYP450 protein has been determined spectrally and a significant enhancement in the protein expression was witnessed in fishes of the experimental groups exposed to the sub-lethal concentrations of lambda water. The spectrally determined microsomal content of CYP450 reveals cyhalothrin when compared to the control group of fishes reared in fresh neither the different isoenzyme composition nor any feature pertaining to a specific isoenzyme. Also the spectrally determined CYP450 content does not indicate the catalytic activity of the enzyme protein. In correlation with the results of the present study, Matsuo et al. (2006) also reported that exposure to crude oil induces CYP1A in fish, *Colossoma macropomum*. George et al., (1995) also showed induction of CYP1A in hepatic tissue of Polar cod, *Boreogadus saida* subjected to dietary crude oil exposure at very low temperatures. Hahn and Stegeman (1994) reported elevated levels of CYP450 1A1 mRNA, protein and catalytic activity (EROD) in fish following various doses of 2.3.7.8-tetra chlorodibenzofuran. Thus the induction of CYP450 in the hepatic tissue of fishes exposed to sub-lethal concentrations of lambda cyhalothrin in the present study reveals the increased activity of the hepatic cells in the detoxification processes.

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TABLES

Table 1a: Effect of lambda cyhalothrin at higher sub-lethal concentration (5.768 ppm) on activity of marker enzymes, gluconeogenic enzymes and cytochrome P450 protein content in liver tissue of *Clarias batrachus*

Enzyme	F Value	P Value	Control	Experimental Days			
				15	30	45	Recovery
ALT	375.21	0.000**	3.892^{a}± 0.103	4.515^{b} ± 0.090 (+16.00)	5.600^{c}± 0.093 (+43.88)	6.262^{d} ± 0.111 (+60.89)	4.332^{b}± 0.192
AST	118.71	0.000**	1.625^{a}± 0.088	1.715^{a}± 0.089 (+5.53)	2.003^{b}± 0.119 (+23.26)	2.942^{c}± 0.123 (+81.04)	1.935^{b}± 0.158

ACP	279.62	0.000**	0.261^{d}± 0.012	0.113^{b}± 0.009 (-56.79)	0.090^{a}± 0.010 (-65.51)	0.088^{a}± 0.008 (-66.28)	0.142^{c}± 0.013
ALP	1117.15	0.000**	0.576^{e}± 0.010	0.284^{c}± 0.007 (-50.69)	0.195^{b}± 0.011 (-66.14)	0.151^{a}± 0.013 (-73.78)	0.346^{d}± 0.017
PHOS	116.93	0.000**	2.813^{a}± 0.109	2.813^{a}± 0.112 (+0.00)	3.260^{c}± 0.133 (+15.89)	4.085^{d}± 0.136 (+45.21)	3.050^{b}± 0.102
PEPCK.	303.16	0.000**	4.485^{a}± 0.197	6.320^{b}± 0.276 (+40.91)	8.665^{d}± 0.232 (+93.20)	7.202^{c}± 0.169 (+60.50)	6.028^{b}± 0.193
PC	783.95	0.000**	30.295^{a}± 0.225	35.537^{c}± 0.260 (+17.30)	39.345^{e}± 0.282 (+29.87)	37.078^{d}± 0.268 (+22.40)	34.515^{b}± 0.404
GGT	96.62	0.000**	1.080^{a}± 0.190	2.005^{bc}± 0.142 (+85.65)	2.752^{d}± 0.090 (+154.81)	2.087^{c}± 0.189 (+93.29)	1.782^{b}± 0.112
CYP450	905.87	0.000**	3.712^{a}± 0.124	4.195^{b}± 0.085 (+13.01)	7.430^{e}± 0.143 (+100.16)	6.800^{d}± 0.111 (+83.18)	4.513^{c}± 0.191

Table 1b: Effect of lambda cyhalothrin at lower sub-lethal concentration (2.884 ppm) on activity of marker enzymes, gluconeogenic enzymes and cytochrome P450 protein content of *Clarias batrachus*

Enzyme	**F Value**	**P Value**	**Control**	**Experimental Days**			
				15	**30**	**45**	**Recovery**
ALT	126.08	0.000**	3.882^{a}± 0.146	4.247^{b}± 0.162 (+9.40)	4.825^{c}± 0.154 (+24.29)	5.723^{d}± 0.138 (+47.42)	4.250^{b}± 0.182
AST	40.89	0.000**	1.555^{a}± 0.173	1.888^{b}± 0.193 (+21.41)	2.410^{c}± 0.177 (+54.98)	2.592^{c}± 0.162 (+66.68)	1.880^{b}± 0.163
ACP	120.23	0.000**	0.269^{d}± 0.015	0.195^{c}± 0.014 (-27.41)	0.137^{b}± 0.012 (-49.07)	0.105^{a}± 0.014 (-60.96)	0.194^{c}± 0.016
ALP	564.62	0.000**	0.570^{e}± 0.014	0.354^{d}± 0.012 (-37.89)	0.242^{b}± 0.017 (-57.54)	0.197^{a}± 0.012 (-65.43)	0.328^{c}± 0.019
PHOS	14.91	0.000**	2.828^{a}± 0.069	2.802^{a}± 0.102 (-0.91)	3.028^{bc}± 0.061 (+7.07)	3.113^{c}± 0.089 (+10.07)	2.910^{ab}± 0.093
PEPCK.	75.184	0.000**	4.578^{a}± 0.309	6.128^{c}± 0.352 (+33.89)	7.480^{d}± 0.262 (+63.39)	6.230^{c}± 0.196 (+36.08)	5.382^{b}± 0.371
PC	451.02	0.000**	30.192^{a}± 0.328	33.825^{c}± 0.411 (+12.03)	39.058^{c}± 0.462 (+29.37)	34.487^{d}± 0.249 (+14.24)	32.263^{b}± 0.412
GGT	20.01	0.000**	1.087^{a}± 0.104	1.883^{b}± 0.149 (+54.83)	2.757^{c}± 0.714 (+153.63)	1.843^{b}± 0.180 (+69.55)	1.360^{ab}± 0.180
CYP450	292.65	0.000**	3.662^{a}± 0.175	3.938^{b}± 0.185 (+7.53)	6.233^{d}± 0.137 (+70.20)	4.707^{c}± 0.116 (+28.53)	3.765^{ab}± 0.144