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Alterations in the carbohydrate metabolism during Deltamethrin -induced toxicity in *Ciprinus carpio*

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Abstract

The aim of this study is to investigate the toxic effect of two different concentrations of (5ppm, 10ppm) deltamethrin in *Ciprinus carpio* different tissues i.e Liver, Heart, Kidney and Brain with reference to Carbohydrate metabolism. The fishes were randomly divided into 3 groups having 6 in each group: (1) Control (2) Deltamethrin-induced toxic group (5ppm) (3) Deltamethrin-induced toxic group (10 ppm). Deltamethrin-induced toxic groups increased the glycogen, glucose and lactate contents and decreased the levels of total carbohydrates (TC) and pyruvate (PYR), Lactate Dehydrogenase (LDH), Isocitrate Dehydrogenase (ICDH), Succinate Dehydrogenase (SDH) and Malate Dehydrogenase (MDH) activities in all the fish organs (Liver, Heart, Kidney and Brain). From the results it is presumed that the Deltamethrin-induced alterations in carbohydrate metabolism occurred in different organs of fish.

Key-Words: Deltamethrin, *Ciprinus carpio*, Carbohydrates

Introduction

Deltamethrin, a synthetic pyrethroid pesticide contaminating aquatic ecosystems as a pollutant, Pesticides applied to the land may be washed into surface waters and may kill or at least adversely influence the life of aquatic organisms [1, 2]. The synthetic pyrethroids are less persistent and less toxic to mammals and birds. The synthetic pyrethroid, deltamethrin [(S)α-cyano-3-phenoxybenzyl- (1R)-cis-3-(2.2-dibromovinyl)-2 2-dimethylcyclopropane carboxylate] has found wideacceptability. The pyrethroids are widely used in field pest control, household use, and as veterinary and human pediculicides and are among the most potent insecticides known [3]. The modifying effect of deltamethrin on sodium and potassium channels has been demonstrated in molluscan neurons [4]. The widespread use of these pesticides consequently leads to the exposure of manufacturing workers, field applicators, the ecosystem, and finally the public to the possible toxic effects of these pesticides. Deltamethrin is a fourth generation synthetic pyrethroid pesticide [5]. Pyrethroids have been shown to be neurotoxic and lethal to fish at concentrations 10–1000 times lower than corresponding values for mammals and birds [6, 7].

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Deltamethrin and other pyrethroids have been found to be extremely toxic to fish [8,9,10,11,12]. Toxicity is highly dependent on stereochemical structure. Most products however, are mixtures of isomers. Pyrethroids are especially advantageous for use in northern climate zones, since they exhibit anegative temperature coefficient of toxicity. They are also considered as relatively non-persistent therefore are not expected to biomagnifies through the food chain.

Hence, the present study is undertaken to examine the toxic effect of different concentrations of deltamethrin on selected biochemical parameters in functionally different types of fish organs with particular reference to carbohydrate metabolism.

Material and Methods

Experimental animals

The tests were performed in a concrete holding tanks, glass aquaria, constant supply of water and good lighting system. The indoor tanks were filled with tap water and aerated for 3 days to help reduce the chlorine content. About 300 active test specimens ranging between 5 and 10 cm standard length were transported to the laboratory from a farm. The specimens were acclimatized to laboratory conditions for 7 days in the indoor holding tanks. The pH, dissolved oxygen concentration and temperature of water in the tanks were monitored.

Preliminary tests were conducted to provide guidance on range of concentration of pesticide to use in the

bioassay. A stock solution of 25 mg/l was prepared from the original product concentration of 12.5 g/l. From the stock solution, the test solutions were prepared using distilled water. The specimens were not fed a day prior to and during toxicity tests to reduce faecal and excess food contaminating the test solution. The nominal test concentrations were 5ppm&10ppm with six replicates each. The results from the toxicity tests were analyzed, using a World Health Organisation (WHO) Computer Pro-gramme, Probit (1982). The concentrations used were converted by the programme to log dose and the number of dead fishes to mortality Probit values. A plot of these two parameters was made from which the LC50 was estimated.

The fishes were maintained according to the ethical guidelines for animal protection and welfare bearing the CPCSEA 438/01/a/cpcsea/dt 17.07.2001 in its resolution No: 9/IAEC/SVU/SK/2009/dt 14.03.2009.

Selection of Pesticide

Deltamethrin, a synthetic pyrethroid pesticide, was selected for the present study. It was obtained as commercial grade chemical from Sigma chemicals, USA.

Experimental design

The fishes were divided into 3 groups, each consisted of 6 and used for studying the effects of different concentrations of deltamethrin.

Group 1 - Control
Group 2 - 5ppm concentration
Group 3 - 10ppm concentration

Isolation of Tissues

The animals were sacrificed. Functionally different organs such as Liver, Heart, Kidney and Brain were separated and frozen in liquid nitrogen (-180^{0}C) and stored at -40^{0}C until further use. At the time of analyses the tissues were thawed and selected parameters were estimated by employing standard methods.

Procurement of Chemicals

All chemicals used in the present study were Analar grade (AR) and obtained from the following scientific companies: Sigma (St. Louis, MO, USA), Fisher (Pittsburg, PA, USA), Merck (Mumbai, India), Ranbaxy (New Delhi, India), Qualigens (Mumbai, India).

Biochemical Analyses

The total carbohydrate content was estimated by the method of Carroll *et al*. (1956)[13]. Glycogen was estimated by the method of Kemp and Van Hejnigen (1954) [14]. Glucose was estimated by the method of Mendal *et al.* (1954) [15]. Lactic acid in the muscle was estimated by the method of Barker and Summerson (1941) [16] as modified by Huckabee (1961) [17]. Pyruvate content of the muscle was estimated by the method of Friedmann and Hangen (1942) [18]. The activity levels of Lactate (LDH), succinate (SDH), and malate (MDH) dehydrogenases were estimated by the method of Nachlas *et al.* (1960) [19] with slight modifications as described by Prameelamma and Swami (1975) [20]. Isocitrate dehydrogenase activity was assayed by the method of Korenberg and Pricer (1951) [21] as modified by Mastanaiah *et al*. (1978) [22].

Statistical treatment of data

All assays were carried out with six separate replicates from each group. The mean, standard error (SE) and Analysis of Variance (ANOVA) were done using SPSS statistical software for different parameters. Difference between control and experimental assays was considered as significant at $P<0.05$.

Results and Discussion

Different parameters of glycolytic and oxidative pathways were estimated in different organs of fish during deltamethrin toxicity. Total carbohydrates were decreased in all the organs during deltamethrin toxicity. Glycogen and glucose levels were elevated in all the organs.

Increased lactate and decreased pyruvate levels were recorded in all the organs during deltamethrin toxicity. Decreased Lactate dehydrogenase (LDH), Isocitrate dehydrogenase (ICDH), Succinate dehydrogenase (SDH) and Malate dehydrogenase (MDH) activities were recorded. Table (1-4). Fish utilizes carbohydrates as the major source of energy for mechanical activity and kinesiological efficiency of the animal. Carbohydrates play not only a structural role in the cell but may serve as a reservoir of chemical energy. Carbohydrates are the major sources of energy fuels for metabolic process readily assimilable, though fats yield more energy [23]. The immediate source of energy for ATP and these biological currencies are replenished ultimately by carbohydrates or fats or proteins.

Selected parameters of glycolytic and oxidative pathways of carbohydrate metabolism were studied in different organs of fish during deltamethrin toxicity. The decrease in total carbohydrate levels in the liver, heart, kidney and brain of deltamethrin treated fishes indicates utilization of carbohydrates to meet energy demands.

The glycogen levels were increased in different organs of treated animals which indicate possible mobilization of stored reserves and mobilization of glycogen from liver to the skeletal muscle in order to meet the energy demands. On par with the glycogen, glucose levels

were also increased in all the organs during deltamethrin toxicity which might be implicated to the increased conversion of glycogen to glucose for the onward glycolytic pathway.

Lactate is the end product of glycolysis under anaerobic conditions and the rate of lactate production is considered as an index of physiological stress in the biological systems [24,25,26]. The lactic acid production and accumulation suggest the tissue capacity to withstand anaerobiosis. The levels of lactic acid also indicate the prevalence of anaerobiosis in the tissues and the tissue specific resistance or susceptibility to anaerobic conditions. In the present study lactate levels were increased in deltamethrin toxic condition. Increased lactate content during toxic condition suggests induction of lacticacedemia in different organs of fish.

The formation of pyruvate, an important end product of glycolysis was found to be low during 5ppm and 10ppm deltamethrin toxicity indicating greater mobilization of pyruvate to lactate through reverse pathway of $NADH_2$ dependent lactate dehydrogenase. The decreased levels of pyruvate and elevated levels of lactate during toxic indicate prevalence oxygen deficiency in the intracellular milieu with advancement of treatment.

NAD-Lactate dehydrogenase (LDH) is a key enzyme of glycolysis and catalyses the reversible oxidation of lactate to pyruvate in the terminal step of glycolysis. The reaction catalyzed by LDH interlinks anaerobic and aerobic oxidation of glucose. The activity of LDH was significantly decreased in all the organs of fish during deltamethrin toxic condition when compared to their respective controls indicating down regulation of oxidative metabolism due to lesser feeding of pyruvate into the TCA cycle.

The reduced levels of oxidative enzymes of TCA cycle i.e. ICDH, SDH and MDH during deltamethrin toxic condition indicate depressed oxidative metabolism in mitochondria and reduced turnover of carbohydrates and energy output [27]. The decreased activities of mitochondrial enzymes could also be attributed to the low feeding and/or availability of substrates, loss of structural integrity of mitochondria and prevalence of hypoxic condition ultimately leading to energy crisis during deltamethrin toxicity. The present findings demonstrate that the alterations in carbohydrate metabolism during toxic effect of different concentrations of deltamethrin on selected biochemical parameters in functionally different types of fish organs such as liver, heart, kidney and brain.

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References

1. W.J. Hayes Jr., E.R. Laws Jr., Handbook of Pesticide Toxicology (3-Volume Set), Academic Press, San Diego, 1991, p. 1576.
2. I.R. Hill, Aquatic organisms and pyrethroids, Pestic. Sci. 27 (1989) 429–465.
3. Smith, T.M., Stratton, G.W., "Effects of synthetic pyrethroid insecticides on nontarget organisms", Res. Rev., 97: 93-119 (1986).
4. T. Kiss, Properties of Na channels in identified snail (Helix pomatia L.) neurones modified by deltamethrin, Pestic.Biochem. Phsiol. 32 (1988) 247–252.
5. J.P. Leahey, The Pyrethroid Insecticides, Taylor & Francis, London, 1985, p. 180.
6. S.P. Bradbury, J.R. Coats, Comparative toxicology of the pyrethroid insecticides, Rev. Environ. Contam. Toxicol. 108 (1989) 133–177.
7. J.T. Eells, J.L. Rasmussen, P.A. Bandettini, J.M. Propp, Differences in the neuroexcitatory actions of pyrethroid insecticides and sodium channel-spesific neurotoxins in rat and trout brain synaptosomes, Toxicol. Appl. Pharmacol. 123 (1993) 107–119.
8. T. Balint, T. Szegletes, Zs. Szegletes, K. Halasy, J. Nemcsok, Biochemical and subcellular changes in carp exposed to the organophosphorus methidation and the pyrethroid deltamethrin, Aquat. Toxicol. 33 (3-4) (1995) 279–295.
9. M. Datta, A. Kaviraj, Acut toxicity of the synthetic pyrethroid deltamethrin to freshwater catfish Clarias gariepinus, Bull. Erviron. Contam. Toxicol. 70 (2) (2003) 296–299.
10. D. Delistraty, Acute toxicity to rats and trout with a focus on inhalation and aquatic exposures, Ecotoxicol. Environ. Saf. 46 (2000) 225–233.
11. Z. Svobodova, V. Luskova, M.J. Drastichova, M. Svoboda, V. Labek, Effect of deltamethrin on haematological indices of common carp

(Cyprinus carpio L.), Acta Vet. Brno. 72 (2003) 79–85.
12. T. Szegletes, T. Balint, Z. Szegletes, J. Nemcsok, In vivo effects of deltamethrin exposure on activity and distribution of molecular forms of carp AchE, Ecotoxicol. Environ. Saf. 31(3) (1995) 258–263.
13. Carroll NV, Longley RW, Roe JH. The determination of glycogen in liver and muscle by use of anthrone reagent. J Biol Chem; 220: 583-93 (1956).
14. Kemp A, Van Heijningen AJ. A colorimetric micro-method for the determination of glycogen in tissues. Biochem J; 56(4): 646-8 (1954).
15. Mendel B, Kemp A, Myers (1954). A colorimetric micro-method for the determination of glucose. BiochemJ; 56: 639–50 (1954).
16. Barker SB, Summerson WH. The Colorimetric determination of lactic acid in biological material. J.Biol. Chem; 138: 535-54 (1941).
17. Huckabee WE. Abnormal resting blood lactate. The significance of hyperlactatemia in hospitalized patients. Am J Med; 30: 840-8 (1954).
18. Friedmann JE, Hangen G. Collection of blood for the determination of pyruvic acid and lactic acids. J. Biol. Chem; 144: 67-77 (1942).
19. Nachlas MM, T Sou KC, Souza E, Cheng CS, Seligman AM. Cytochemical demondtration of succinic dehydrogenase by the use of new p-nitrophenol substituted ditetrazolium; J. Histochem; 5: 420-436 (1960).
20. Prameelamma Y, Swami KS. Gluthathione dehydrogenase activity in normal and denervated gastocnemius muscle of frog, Rana hyxadactyla. Curr. Sci; 44: 739-740 (1975).
21. Korenberg A, Pricer E. Structure of triphosphopyridine nucleotide di and tri phosphopyridine nucleotide isocitrate dehydrogenase in yeast. J. biol. Chem; 186: 557 (1951).
22. Mastanaiah S, Chengal Raju D, Swami KS. Orcadian rhythmic activity of lipase in scorpion, heterometry fulvipes (C. Koch). Curr.Sci; 20(47): 130-131 (1978).
23. Martin FC, Peters TJ. Assesment in vitro and in vivo of muscle degradation in chronic skeletal muscle myopathy of alcoholism. Clin Sci; 66: 693-700 (1985).
24. Mc Cartney N, Heigenhauser GIF, Jones NL. Power out put and fatigue of human muscle in maximal cycling exercise. J. Appl. Physiol;55: 218-224 (1983).
25. Buono MJ, Clancy TR, Cook JR. Blood lactate and ammonium ion accumulation during graded exercise in humans. J. Appl. Physiol: Respirat Environ. Exercise Physiol; 57: 135-139 (1984).
26. Kozlowski S, Brzezinska Z, Kruk B, Kaciubauscilko H, Green leaf JE, Nazar, K. Exercise hyperthermia as a factor limiting physical performance temperature effect on muscle metabolism. J. Appl. Physiol;59: 766-773 (1985).
27. Murray RK, Bender DA, Botham KM, Kennelly PJ, Rodwell VW, Weil PA. Harper's Illustrated Biochemistry, 28th edition, Mc Graw Hill Medical, New York; 2009.

Table 1: Alterations in the Carbohydrate metabolism in Liver of *Ciprinus carpio* during deltamethrin toxic condition

(The values of Total carbohydrates, glycogen, glucose and lactate were expressed as mg/gm wet wt; pyruvate levels expressed as µ moles / gm wet wt. and LDH, ICDH, SDH, and MDH activities were represented as µ moles of formazan formed/mg protein/ hour)

Liver	Control	5ppm	10ppm
TC	10.124	8.605*	7.315*
	±0.010	±0.018	±0.007
		(-15)	(-27.75)
GLY	2.604	3.291*	4.160*
	±0.024	±0.008	±0.012
		(26.4)	(59.77)
GLU	1.684	1.719	2.443*
	±0.054	±0.052	±0.049
		(2.1)	(45.08)
LAC	2.624	2.844	4.221*
	±0.059	±0.067	±0.042
		(8.4)	(60.86)
PYR	16.248	12.348*	9.385*
	±3.069	±2.070	±3.664
		(-24)	(-42.24)
LDH	4.568	3.015*	1.990*
	±0.272	±0.128	±0.059
		(-34)	(-56)
ICDH	1.862	1.650*	1.462*
	±0.021	±0.037	±0.039
		(-11.4)	(-21.5)
SDH	2.864	1.948*	1.324*
	±0.272	±0.128	±0.059
		(-32)	(-53.76)
MDH	2.189	1.681*	1.291*
	±0.043	±0.017	±0.029
		(-23.2)	(-41.18)

All the values are mean, ±SE of six individual observations. Values in '( )'parentheses are % change over Control control.
*Values are significant at P < 0.05 in Scheffe test.

Table 2: Alterations in the Carbohydrate metabolism in Heart of *Ciprinus carpio* during deltamethrin toxic condition

(The values of Total carbohydrates, glycogen, glucose and lactate were expressed as mg/gm wet wt; pyruvate levels expressed as µ moles / gm wet wt. and LDH, ICDH, SDH, and MDH activities were represented as µ moles of formazan formed/mg protein/ hour)

Heart	Control	5ppm	10ppm
TC	8.106	6.080*	5.168*
	±0.020	±0.017	±0.016
		(-25)	(-36.25)
GLY	2.226	2.760*	3.489*
	±0.008	±0.009	±0.003
		(24)	(56.73)
GLU	1.346	1.617*	2.265*
	±0.008	±0.009	±0.003
		(20)	(68.26)
LAC	2.456	3.537*	3.708*
	±0.029	±0.020	±0.011
		(44)	(50.97)
PYR	11.226	8.083*	6.143*
	±8.606	±2.427	±3.069
		-(28)	(-45.28)
LDH	4.128	2.642*	1.744*

	±0.008	±0.009	±0.003
		-(36)	(-57.76)
ICDH	1.648	1.417*	1.256*
	±0.030	±0.012	±0.016
		(-14)	(-23)
SDH	2.662	1.704*	1.159*
	±0.008	±0.009	±0.003
		(-36)	(-56)
MDH	1.886	0.877*	0.674*
	±0.043	±0.017	±0.029
		(-53)	(-64)

All the values are mean, ±SE of six individual observations. Values in '( )'parentheses are % change over Control control. *Values are significant at P < 0.05 in Scheffe test.

Table 3: Alterations in the Carbohydrate metabolism in Kidney of *Ciprinus carpio* during deltamethrin toxic condition

(The values of Total carbohydrates, glycogen, glucose and lactate were expressed as mg/gm wet wt; pyruvate levels expressed as µ moles / gm wet wt. and LDH, ICDH, SDH, and MDH activities were represented as µ moles of formazan formed/mg protein/ hour)

Kidney	Control	5ppm	10ppm
TC	6.248	4.499*	3.824*
	±0.029	±0.020	±0.011
		(-28)	(-38.8)
GLY	1.854	2.325*	2.939*
	±0.043	±0.017	±0.029
		(25.4)	(58.5)
GLU	1.062	1.488*	1.817*
	±0.029	±0.020	±0.011
		(40.1)	(71.06)
LAC	2.224	3.305*	3.582*
	±0.021	±0.037	±0.039
		(48.6)	(61.082)
PYR	18.249	12.044*	9.154*
	±5.606	±2.427	±3.069
		(-34)	(-49.84)
LDH	3.648	2.284*	1.507*
	±0.272	±0.128	±0.059
		(-37.4)	(-58.684)
ICDH	1.442	1.242*	1.100*
	±0.008	±0.009	±0.003
		(-13.9)	(-23.715)
SDH	2.458	1.494*	1.016*
	±0.043	±0.051	±0.024
		(-39.2)	(-58.656)
MDH	1.624	0.914*	0.702*
	±0.043	±0.017	±0.029
		(-43.7)	(-56.762)

All the values are mean, ±SE of six individual observations. Values in '( )'parentheses are % change over Control control.*Values are significant at P < 0.05 in Scheffe test.

Table 4: Alterations in the Carbohydrate metabolism in Brain of *Ciprinus carpio* during deltamethrin toxic condition

(The values of Total carbohydrates, glycogen, glucose and lactate were expressed as mg/gm wet wt; pyruvate levels expressed as µ moles / gm wet wt. and LDH, ICDH, SDH, and MDH activities were represented as µ moles of formazan formed/mg protein/ hour)

Brain	Control	5ppm	10ppm
TC	4.864	3.988*	3.390*
	±0.031	±0.016	±0.018
		(-18)	(-30.3)
GLY	1.402	1.738*	2.197*
	±0.010	±0.006	±0.008
		(24)	(56.736)
GLU	0.986	1.184*	1.659*
	±0.010	±0.006	±0.008
		(20.1)	(68.26)
LAC	2.046	2.946*	3.194*
	±0.011	±0.007	±0.068
		(44)	(56.096)
PYR	12.654	9.124*	6.934*
	±2.455	±2.093	±2.448
		(-27.9)	(-45.204)
LDH	3.247	2.163*	1.427*
	±0.010	±0.006	±0.008
		(-33.4)	(-56.044)
ICDH	1.226	0.569*	0.504*
	±0.043	±0.051	±0.024
		(-53.6)	(-58.89)
SDH	2.126	1.293*	0.879*
	±0.010	±0.006	±0.008
		(-39.2)	(-58.656)
MDH	1.224	0.450*	0.346*
	±0.028	±0.017	±0.023
		(-63.2)	(-71.738)

All the values are mean, ±SE of six individual observations. Values in '( )'parentheses are % change over Control control.
*Values are significant at $P < 0.05$ in Scheffe test.

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