Research Article

ACETYLCHOLINE ESTERASE, ACETYLCHOLINE, SUCCINIC DEHYDROGENASE AND LACTIC DEHYDROGENASE CHANGES IN THE FRESH WATER FOOD *FISH CHANNA STRIATA* AFTER EXPOSURE TO *CLEISTANTHUS COLLINUS* SUICIDAL PLANT EXTRACT

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ABSTRACT

The enzymes Acetylcholinesterase (AchE), Acetylcholine (Ach), Succinic dehydrogenase (SDH) and Lactatedehydrogenase (LDH) are used as biological markers in the present study. Enzymes are highly sensitive and used to evaluate the biological effects of suicidal plant extract *Cleistanthus collinus* in freshwater food fish *Channa striata*. A significant increase in the Ach, LDH and SDH activity in muscle, liver and gill were observed. The AchE activity significantly declined by -49.80% liver, -45.00% gills and -38.03% in liver.

Keywords: Acetylcholinesterase, Acetylcholine, Succinic dehydrogenase, Lactatedehydrogenase, *Cleistanthus collinus, Channa striata*.

INTRODUCTION

Biochemical and physiological indicators such as enzymes can be used to identify possible environmental problems before the health of aquatic systems is seriously altered[1]. The measurement of enzymes concentration is a classical means by which the health of fish population is assessed in different water sources[2]. The alterations in metabolic rates by the functioning of enzymes at the molecular level form the basis for an organism to self regulate. Enzyme inhibition is a common mechanism of toxicity[3], although toxicity is dependent on the magnitude of inhibition and the concentration of the enzyme present. Specific enzymes regulating variety of metabolic pathway can be altered as a result of stress related homeostatic adjustments induced by toxicant exposure[4].

Acetylcholine is released from pre-ganglionic neurons of parasympathetic division of autonomic nervous system. It is a unanimously accepted fact that hydrolysis of acetylcholine (ACh) to choline and acetic acid is catalyzed by enzyme cholinesterase in animal system. The enzyme prevents accumulation of excessive acetylcholine at cholinergic synapse and at neuromuscular junction[5]. Acetylcholinesterase (AchE) is an enzyme that modulates the amount of neurotransmitter substance acetylcholine (Ach) at neuron junctions[6] and inhibition of AChE activity was regarded as significant parameters in assessing complex toxicogenic effects of various toxicants[7,8].

Dehydrogenase are the enzymes involved in the energy release by the biological oxidation of food stuff inside the mitochondria, and also in the production of reduced potential (NADPH) required in the biosynthetic and detoxification mechanisms. When the fish are exposed to environmental stress, alterations in the activity of the dehydrogenase and reduced potential (NADPH) were observed[9]. LDH is an enzyme associated with anaerobic pathway of carbohydrate metabolism. Lactic acid, a measure of anaerobic metabolism has been widely used and increase of anaerobic metabolism have been shown to be a rapid and clear response of depletion of energy caused by lack of oxygen[10]. The cytoplasmic enzyme LDH is generally associated with cellular metabolic activity. It acts as a pivotal enzyme between the glycolytic pathway and the tricarboxylic acid cycle. Lactate is the end product of the glycolytic sequence under anaerobic condition. According to Natarajan[10] an upward trend in lactic acid in the tissues may be taken to suggest that oxygen supply to the tissues is not adequate for the normal metabolic function.

SDH is an oxidative enzyme. It is the active regulatory enzyme of the tricarboxylic acid cycle. It is an active parent enzyme of the TCA involved in the central oxidative pathway for carbohydrates, fats as well as most of the aminoacids. SDH being a metalloprotein bound to the inner surface of the inner mitochondrial membrane (unlike the Kreb cycle enzymes which are part of the matrix) involved in the direct transfer of hydrogen atom from substrate to a flavoprotein without the participation of NAD^+. SDH catalyses the dehydrogenation of succinate to fumerate involving flavin adenine dinucleotide (FAD). The tightly bound FAD can be reoxidized only by linking the SDH holoenzyme to the respiratory chain enzymes, which are also part of inner membrane[11]. This is the only dehydrogenase step in the citric acid cycle which is not NAD linked[12]. Any disturbance in the enzyme activities is bound to reflect in the TCA cycle operation and inturn influence the alternate pathway of carbohydrate metabolism[13]. The maintenance of energy requirements on adaptation to meet the depressive effects[14]. The succinate dehydrogenase activity has been reported earlier in gold fish gill[15].

The protein content in the tissues of animals plays a role in the metabolism of animals[16]. The soluble protein fraction represents the activity level of enzymes in

general. The structural protein fraction forms the structural moiety of a cell[17]. Depletion protein in the fish indicates the physiological strategy in order to meet the energy demand and to adapt itself to the changed metabolic system which may lead to the stimulation of degradative processes like proteolysis and utilization of degraded products for increased energy metabolism[18]. In general, organophosphorus and organochlorine pesticides are known to depress blood protein in fishes[19,20]. Many plant extracts act as biopesticides and fish poisons[21]. *Clcistanthus collinus* leaf extract is widely used in Dharmapuri district for suicide purposes. Fish being the inhabitant of closed environment becomes a useful model in assessing the effect of plant extracts on biochemical and physiological parameters. The present paper deals with the effect of suicidal plant extract on some selected enzyme systems in different tissues of the freshwater food fish, *Channa striata*.

MATERIALS AND METHODS

Fish (10-15g) collected from local freshwater sources were maintained in the laboratory at $28^0 \pm 1^0$C and exposed to a lethal concentration of plant extract (Lc_{50}10mg/l/48 hr) Lc50 value was calculated by Probit method[22]. After exposure, the gill, liver and muscle tissues were analyzed for the acetylcholinesterase[23], acetylcholine[24], Succinate dehyrogenase and lactate activities[25], and the protein estimation[26]. Statistical significance of difference between control and treated groups of different exposure period were tested by using 't' test [27].

RESULT AND DISCUSSION

Acetylcholine is the major transmitter substance in vertebrates. It is an ammonium compound. The arrival of nerve impulses at the synaptic knob depolarizes presynaptic membrane, causing calcium channels to open, increasing the permeability of the membrane to calcium (Ca2+) ions[28]. AchE is an enzyme that is essential for the normal functioning of the central and peripheral nervous system[29] and is widely distributed in the neural and non-neural tissues[30]. There is a significant reduction in the AchE activity in all the three tissues of the experimental fish and is in the following order: Muscle > Gill > liver. Concomitant with the AchE inhibition, there is an accumulation of Ach in the tissues of plant extract treated fish (Table-1). The accumulation is more in the muscle followed by liver and gill. Inhibition of AchE in these tissues may cause functional imbalance and structural disintegration in these organs. Earlier studies of AchE activities in the tissues of *Periplaneta Americana* exposed to Fenvalerate disclose the same trend observed in the present investigation[31]. Inhibition of this enzyme in the liver may due to the synergistic action of parent compound and its metabolites which are formed as a result of biodegradation[32]. An observed increase in Ach content consequent to decrease in the tissue[33,34]. AchE level in *Tilapia mossambica* exposed to malathion. Similar inhibition of ache in the brain tissue of fish exposed to malathion[35]. Decrease in acetylcholinesterase activity in *Labeo rohita* exposed to malathion[36]. Inhibition of AchE with concomitant increase in ach content in the tissue of *Cyprinus carpio* exposed to fenvalerate[37]. They also reported that this is an implication of greater inhibition in the inhibitory activity of the neural nervous system and Ach accumulated in brain and other tissues.

Table 1: Effect of suicidal plant extract on AchE activity, ACH content, SDH and LDH activities of Chosen organs of *Channa striata*

Tissue	AchE activity		Ach content		SDH activity		LDH activity	
	Control	Experimental	Control	Experimental	Control	Experimental	Control	Experimental
Gill	42.11± 2.81	23.16 ± 2.01	14.06 ±1.46	27.21± 1.92	0.042 ± 0.001	0.010 ± 0.001	0.008 ± 0.002	0.013±0.001
% change	-45.00		+93.53		- 76.19		+62.50	
Liver	25.82 ± 2.00	16.00 ± 1.12	12.18±2.60	26.12 ± 3.00	0.17 + 0.01	0.05 ± 0.002	0.05 ± 0.001	0.09 ± 0.006
% change	-38.03		+114.45		-70.59		+80.00	
Muscle	58.01± 3.70	29.12 ± 3.10	6.75± 2.80	19.00 ± 2.01	0.06 ±0.002	0.06 ±0.002	0.008 ± 0.002	0.02 ± 0.003
% change	-49.80		+181.48		-66.67		+150.00	

a μ mole Ach hydrolysed / mg protein / hr; b μ mole / g wt; c μ mole formzan formed / mg proteins / hr; Values are mean ± S.E of 6 observations and significant at $P < 0.05$.

Succinic dehydrogenase (SDH), an important enzyme of the Kreb's cycle of mitochondria is encoded on the fragment of chromosome 1. In, fact, SDH contains three different kinds of iron-sulphur clusters, embedded in the inner mitochondrial membrane. In the mitochondrial membrane, SDH unite with ubiquinone from succinicubiquinone reductase, the so-called complex II of the respiratory chain. SDH is involved in Kerb's cycle and catalyzes succinic to fumarate. Along with brain cells, this reaction is important in nearly all mammalian cells[38]. SDH is significantly inhibited in all the organs of experimental fish. Gill is more vulnerable than other organs. The inhibition of activity SDH also reflects the rate of TCA cycle resulting in the decreased synthesis of high energy phosphatase like ATP and ADP. The Similar decrement in the SDH activity was also observed by the various workers in different organism exposed to the toxicants[39]. A significant decrease in the activity on succinate dehydrogenase activity in muscle, liver and brain tissue of fish *Cyprinus carpio* exposed to different sublethal concentrations of distillery effluent[40]. The lactate dehydrogenase is an anaerobic enzyme involved in the conversion of pyruvate to lactate in the Embden Meyerhoff pathway. The increase LDH activity in the present study is attributed to the conversion of accumulated pyruvate[41] into lactate[42,43] which is transported through blood to liver and reconverted glucose and glycogen to meet energy need under physiological stress. Increased malate dehydrogenase and lactate dehydrogenase activities in the crab

Oziotelphusa senex exposed to sumithion, an organophosphate insecticide[44]. Similar increase in LDH and alkaline phosphatase activities were observed in the English sole *Parophrys vetulus* treated with carbon tetrachloride[45]. Also, cadmium was reported to have elicited increased muscular LDH activity in Fiddler crab, *Uca pugilator*[46] and in the brook trout, *Salvelinus foninalis*[47]. On the other hand, some agrochemicals and heavy metals inhibit tissue enzymes. Depression of SDH and elevation of LDH strongly indicate favoring of anaerobic metabolism in plant extract stressed fish to meet the energetic demands. Probably, some neurotoxic and oxidoreductive principles are present in the suicidal plant extract which warrants further studies.

REFERENCES

1. Jiminez B.D. & Stegeman, J.J. Detoxication enzyme as indicator of environmental stress on fish. *Am. Fish. Soc. Symp.,* 8, 1990. 67 -79.
2. Landis W.G. & Yu, M. Introduction to environmental Toxicology : impacts of chemicals upon ecological system. Lewis Publishers. Boca Raton. 1995. pp 328.
3. Westlake G.E., Martun A.A., Stanly P.I. & Walker C.H. Control of enzymes levels in the plasma, brain and liver from wild birds and mammals in Britain. Comp. *Biochem. Physiol., 76C,* (1983). 644 – 680.
4. Head E.J.H. & Gabbott P.A. Control of NADP+ Dependent Isocitrate Dehydrogenase activity in the Mussel *Mytilus edulis* L., *Biochem. Soc. Trans.* 7, 1979, 868 – 98.
5. Konar, 1979. Pesticides and aquatic ecosystem. Indian. J. Fish., 22, 1979. 80 -85.
6. O'Brain R.D. Insecticides action and metabolism. Academic press, New York. 1967.
7. Bandyopadhyay R. Inhibition of acetylcholinesterase by peremethrin and its reversion by acetylthiocholine. *Ind. J. Exp. Biol.,* 20, 1982, 488 – 491.
8. Bradbury S.P., Mckim J.M. & Coats J.R. Physiological responses of rainbow trout Salmo gairdneri to acute Fenvalerate intoxication. *Pestic Biochem Physiol.,* 27, 1987, 275 – 288.
9. Saxena P.K., Bedi R. & Soni G.L. Effects of vegetable oil factory effluent on the levels of phosphatases and dehydrogenases in the liver and kidney of the freshwater teleost *Channa punctatus* (BL). *Environ. Pollut.,* 28, 1982, 245 – 253.
10. Natarajan G.M. Studies on some aspects of physiology of South Indian air-breathing fish, *Macropodus cupanus* (Cuv and Val) with special reference to strategies for adapting to changing environmental conditions. *Ph.D. thesis, submitted to Bharathiar University,* Coimbatore. 1987.
11. Hatefi, 1985. The mitochondrial Electron transport and oxidative Phosphorylation system. Ann. Rev. Biochem., 54, 1985, 1015 – 1060.
12. Hochachka, P.W. Isoenzymes in metabolic adaptations of a poikilotherm: subunit relationship in lactic acid dehydrogenase of gold fish. Arch. Biochem. Biophysi. 111, 1969, 96 – 103.
13. Randall M.J., Parry, M.J., Hawkeswood E., Cross P.E. & Dickinson R.P. A novel selective thromboxane synthetase inhibitor with platelet antiaggregatory and antithrombotic activity. Thromb. Res., 23, 1981, 145 – 162.
14. Hochachka & somera, 1973. Strategies of biochemical adaptations. W.B. Saundrs, Philadelphia, USA. 1973, 385.
15. Sexton & Russel, succinic dehydrogenase activity in the Goldfish Gill. Science, 1955, 342 – 343. 1955 Succinic
16. Palanivelu V., Vijayavel K., Ezhilarasibalasubramanian S. & Balasubramanian M.P. Impact of fertilizer (urea) on oxygen consumption and feeding energetics in the fresh water fish *Oreochromis mossambicus.* Environmental Toxicology and Pharmacology, 2, 2004, 351-355.
17. Lehninger A.L. *Biochemistry. Kalyani Publications, Ludhiana, India.* 1978, pp 223 – 236.
18. Begam G. & Vijayaraghavan, S. Alterations in protein metabolism of muscle tissue in the fish *Clarias batrachus* (Linn.) by commercial grade Dimethoate. *Bull. Environ. Contam. Toxicol.,* 57, 1996, 223-228.
19. Grant B.F. & Mehrle P.M. Endrin toxicosis in rainbow trout *(Salmo gairdneri). J. Fish. Res. Board. Can., 30,* 1973, 31 – 40.
20. Mukhopadhyay P.K. and Dehadrai T.V. Studies on air-breathing catfish *Clarias batrachus* (Linn.) under sublethal malathion exposure. *Ind. J. Exp. Biol.,* 18, 1980, 348 – 352 .
21. Parimala, K. M.Phil Dissertation, Bharathiar Univesity, Tamil Nadu, India, (2002).
22. Finney D.J. Probit Analysis, Cambridge Univ. Press, London. 1964.
23. Metcalf R.L. In Methods in Biochemical analysis edited by D.Glick (Inter Science publishers, Inc, New York, 1957.
24. Angustinsson K.B. In Methods in Biochemical analysis, edited by D. Glick (Inter Science publishers, Inc, New York). 1957.
25. Nachlas M.M., Margulies, S.P. & Seligman, *A.M. J. Biol.chem.*, 235, 1960. 2735.
26. Lowry O.H., Rosebrough N.J., Farr, A.L. & Randall R.J. *J. Biol. Chem.*, 193, 1951, 265.
27. Zar J.H. *Biostatistical Analysis* (2nd Ed.) Prentice Hall Inc., New Jersey, U.S.A. 1984.
28. Mitchell K.M. Acetycholi ie and choine amperometric enzyme sensors characterized *in vitro* and *in vivo*. *Anal. Chem.,* 76 (4), 2004. 1098-1106.
29. Hart A.D.M. Relationship between behaviour and the inhibition of acetylcholinesterase in birds exposed to organophosphorus pesticide. *Environmental Toxicology and Chemistry.,* 12, 1993. 321 – 336.
30. Friboulet A., Reiger F., Goudou D., Amitai G. & Taylor P. Interaction of an organophosphate with a peripheral site on acetylcholinesterase. *Biochemistry.,* 29, 1990, 914 – 920.
31. Yellamma K. & Ravikumar A. Behavioural and physiological changes in the cockroach, Periplaneta Americana exposed to Fenvalerate. *Indian. J. Exp. Biol.,* 25: 1987, 256 – 261.
32. Casida J.E., Gammon D.W., Glickman A.H. & Lawrence L.J. Mechanisms of selective action of pyrethriod insecticides. *Ann Rev Pharmacol Toxicol.*, 23: 1983. 413 – 438.

33. Sahib I.K. & Ramana Rao K.V. Correlation between subacute toxicity of malathi in and AchE inhibition in the tissues of the teleost, *Tilapia mossambica. Bull. Environ. Contam. Toxicol.,* 24, 1980a. 711- 718.

34. Sahib I.K. & Ramana Rao, K.V., Toxicity of malathion to the freshwater fish, *Tilapia mossambica*. *Bull. Environ. Contam. Toxcol.,* 24, 1980b, 870-874.

35. Coppage D.L., Mathews E., Cook G.H. & Knight J. Brain AchE exhibition as diagnosis of environmental poisoning by malathion, 0-0 S (1,2 dicarbody ethyl phosphorodi-thioate). *Pest. Biochem.,* 5, 1975, 536.

36. Singh M. & Kumar S. Acetycholinestease activity and enzyme kinetics in the brain of freshwater telecost, *Catla catla* (Ham) subjected to subchronic and actue exposure to malathion. *U.P.J. Zool.,* 20, 2000. 01-06.

37. Reddy M.M., Anandkumar V., Reddy P.S. & Reddy S.L.N. Phenol induced metabolic alterations in the brain and muscle of a freshwater *Channa punctatus* during sublethal toxicosis. *J. Ecotoxicol. Environ. Monit*., 3(1), 1993, 13-17.

38. Stachowska E., Bober J., Chlubek D. & Machoy Z. Number of fluoride ions binding of succinate dehydrogenase during mixed inhibition. *Fluoride.,* 33 (3), 2000, 115 – 120.

39. Sudharsan R., Shobha R.A., Reddy T.N., Reddy P.U.M. & Raju T.N. Effect of nitrite toxicity on the dehydrogenase in the fish *Tilapia mossambica*. *J. Environ. Poll.,* 1, 2000, 127 – 130.

40. Ramkritinan C.M., Kumaraguru A.K. & Balasubramanian M.P. Impact of distillery effluent on carbohydrate metabolism o freshwater fish *Cyprinus carpio*. *J. Fish Biol.,* 2005. 14 : 693.

41. Reichl M., Khan M.A. & Sleet R.A. Diagnosis of acute bone pain using isotope bone imaging. Archives of Emergency Medicine, 5, 1987, 169- 172.

42. Anuradha C.H. & Raju T.N. Effect of Selenium toxicity of dehydrogenases in the fish *Anabas scandens*. *J. Ecotoxico. Envn. Monit.,* 6(2), 1996. 149 – 151.

43. Mary Chandravarthy V. Biochemical alterations of freshwater fish Anabas scandens (Cuvier) during exposure and recovery periods after lead nitrate and lead acetate intoxication. Ph.D thesis. Osmania University. Hyderabad, 1990.

44. Reddy M.M., Anandkumar V., Reddy P.S. & Reddy S.L.N. Phenol induced metabolic alterations in the brain and muscle of a freshwater *Channa punctatus* during sublethal toxicosis. *J. Ecotoxicol. Environ. Monit*., 3(1) 1983, 13-17.

45. Casillas E. & Ames, W. Hepatoxic effects of carbon tetrachloride on the English sole (*Parophrys vitulus*): possibleindicator of liver dysfunction. *Comparative Biochemistry Physiology.,* 84C, 1986, 397 – 400.

46. Devi M., Reddy P.S. & Fingerman M. Effect of exposure on lactate dehydrogenase activity in the hepatopancreas and abdominal muscles of the Fiddler crab Uca pugilator. 1993.

47. Christensen G., Munt E. & Fiandt, J. The effect of methyl mercuric chloride, cadmium chloride and lead nitrate on six biochemical factors of brook trout (*Salvelinus fontinalis*).*Toxicology and Pharmacology.*, 42, 1977, 523 – 530.

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