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Toxicity of chlorpyrifos on protease and glutamate dehydrogenase enzyme activities in albino rats

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Abstract: Present study was aimed to elucidate the pesticide toxicity in rats involves induced abnormalities of the intracellular protein catabolic process by the effect of one of the commonly used organophosphate compound chlorpyrifos on the activities of representative protein catabolising proteases and Glutamate dehydrogenase (GDH) is a one of the regulatory enzyme known to check the deamination process to minimize the ammonia level and plays a significant role in the catabolism of amino acids. The sub lethal stress of chlorpyrifos on important metabolites and enzymes of protein metabolism was investigated in most important tissues like liver, kidney, heart and intestine of albino rats. Sub lethal concentration (1/10th LD_{50} i.e., 20mg/kg body weight) of chlorpyrifos (Organophosphate) on the enzyme parameters of albino rats were analysed after single, double and multiple dose of exposure. The increased protease activities in the different tissues of rat indicate the damage caused due to impairment of energy supply and proteases activity indicates higher protein degradation. Therefore, the proteins are denatured leading to more activation of proteases. The elevated GDH activity levels indicate its contribution to ammonia production and glutamate oxidation during chlorpyrifos toxicity.

Key words: Chlorpyrifos; Protease; GDH; Albino rat.

Introduction

The use of pest control chemicals has increased several folds in India and is likely to increase in the forthcoming years. It is a well-known fact that indiscriminate use of pest controls in agriculture has resulted in widespread distribution in the environment and also has a direct and indirect impact on non-targeted organisms. Pesticides can move from the site of application via drift, leaching, and runoff, which have various characteristics that determine how they act once in soil. Some commonly used pest chemicals (pesticides) persistent measurable residues in soil from three to five years[1,2]. Indiscriminate use of different pesticides in agriculture to prevent crop damage from pests has increased over the years, especially in the developing countries[3]. In 1959, it was estimated that about 50,000 of them had been synthesized[4]. Nowadays more than 1,00,000 different organophosphorus compounds have been synthesized and their insecticidal properties evaluated[5]. Among them organophosphate (OP) pesticides are widely used because of their biodegradability[6].

Protease is an enzyme that conducts proteolysis that begins protein catabolism by hydrolysis of the peptide bonds that link amino acids together in the polypeptide chain. Proteases are known to breakdown proteins to small peptides and ultimately to amino acids. They are present in almost all the tissues of mammals[7]. The proteases with neutral pH as optimum are associated with peroxisomes and lysosomes referred as neutral proteases[8,9]. Among the proteases some are lysosomal in origin having acidic pH optimum, which are generally termed as cathepsins[10]. Besides these two types, other type of protease with an alkaline pH optimum was detected in cytosolic fraction generally called as alkaline protease[11]. Increase in acidic protease activity may be due to increase in number and size of lysosomes, neutral proteases causing structural organization in different tissues and causes disassembly of intact myofibrils during metabolic turnover of myofibrillar proteins[12]. The changes in protease activities indicate the changes in energy cycle. All the proteins under normal conditions, irrespective of their location, are continuously degraded and replaced by new ones[13]. Proteolytic activity is known to increase in various physiological and pathological conditions[14].

Glutamate dehydrogenase (GDH) is a regulatory enzyme known to check the deamination process to minimize the ammonia level and plays a significant role in the catabolism of amino acids. These enzymes function as a link between protein and carbohydrate metabolisms and the net outcome is the incorporation of keto acids into the TCA cycle. There is much evidence for the shifts in the activities of these enzymes to a variety of environmental and physiological conditions[15].

Glutamate dehydrogenase enzyme is present in cytoplasm and mitochondria. The cytoplasmic GDH recycle the cytoplasmic origin of ammonia and keeps up glutamate level for mitochondrial transport. Subsequently mitochondrial GDH supplies α-ketoglutarate to Krebs cycle especially when the animal is in stress condition. GDH plays a crucial role in the nitrogen metabolism by functioning both in amino acid catabolism and

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their biosynthesis. GDH allows the incorporation of ammonia into α-ketoglutarate before being transferred by transamination to other α-keto acids[16]. In the present study an attempt has been made to observe the effect of an organophosphate compound chlorpyrifos on Protease and Glutamate enzyme activities in albino rats.

Materials and Methods

Pesticide: Chlorpyrifos Technical (95.30%) was obtained from Nagarjuna Agri. Chem Limited, Ravulapalem Mandal, East Godavari District, A.P., India.

Pesticide stock solution: Stock solution of chlorpyrifos was prepared in acetone. Working pesticide test solutions were prepared by diluting the stock solution with distilled water.

Animal Model: Healthy adult albino rats of same age group (100±10 days) and weight (200 ±10 g) were obtained from the Indian Institute of Sciences (IISc) Bangalore, India. They were kept in well cleaned, sterilized cages and maintained conditions (25±2ºC and with 12 hr light, 12 hr darkness) food and water were allowed *ad libitum*.

Experimental Design:

The toxicity of Chlorpyrifos was evaluated by probit method of Finney[17] and the LD_{50} of chlorpyrifos to albino rats was found to be 200 mg/kg bw. 1/10 of LD_{50} value (20mg/kg bw) was selected as sub lethal dose. The animals were divided in to four groups having ten animals each. The first group animals treated as control animals. Second, third and fourth groups of animals were termed as experimental animals. To the animals of second group single dose of pesticide (i.e. on first day) was administered orally by gavage method. To the third group of animal's double doses were given i.e. on 1st and 3rd day. Similarly, multiple doses i.e. 1st, 3rd, 5th and 7th day were given to the fourth group of animals. After stipulated time the animals were sacrificed and collected the tissues like liver, kidney, heart and intestine for the estimation of antioxidant enzyme activities.

Estimation of Protease activity

Protease activity was estimated by the method of Moore and Stein[18] considering the amount of free amino acids liberated from the protein substances as a measure of proteolytic activity. 4% w/v homogenates were prepared in cold distilled water. The homogenates were centrifuged at 1000rpm for 10 minutes. The supernatant was used as enzyme source. The reaction mixture in a volume of 2 ml contained 100 µ moles of phosphate buffer (pH 7.4), 20 mg of heat denatured hemoglobin as substrate and 0.5ml of the supernatant. The contents were incubated at 37°C for 30 minutes and the reaction was stopped by the addition of 2 ml of 10% TCA. Zero time controls were conducted by adding 2 ml of 10% TCA prior to the addition of enzyme source. The contents of the samples were filtered and the free amino acid level was determined in the filtrates. To 0.5 ml of aliquot of the filtrate, 2 ml of ninhydrin reagent was added. The contents were heated in boiling water bath for 5 minutes and cooled. The volume was made up to 10 ml with distilled water and read at 570 nm against a reagent blank in a spectrophotometer. All the samples were corrected with zero time controls. The proteolytic activity was expressed as µmoles of tyrosine equivalents / mg protein / hr.

Estimation of Glutamate dehydrogenase (GDH) (L-glutamateNAD oxidoreductase; EC=1.4.1.3):

The activity of GDH was assayed by the method of Lee and Lardy[19]. 3% w/v tissue homogenate was prepared in ice cold 0.25M sucrose solution and centrifuged at 1000xg for 15 minutes. The supernatant was used as enzyme source. The reaction mixture in a volume of 2ml contained 100µ moles of phosphate buffer (pH 7.2), 4.0 π moles of sodium glutamate, 0.1 µ moles of NAD, 4 µ moles of INT and 0.2 ml enzyme source. The reaction mixture was incubated at 37°C for 30 minutes and the reaction was stopped by adding 5 ml of glacial acetic acid. Zero-time controls were maintained by adding 5 ml of glacial acetic acid prior to the addition of homogenate. The formazon formed was extracted overnight in 5 ml of cold toluene. The intensity of color developed was read at 495 nm against a reagent blank in a spectrophotometer. The enzyme activity was expressed as µ moles of formazon formed / mg protein / hr.

Results

The results of protease activity in the control and experimental albino rats under the study are given in Table. 1. The experimental rats exposed to chlorpyrifos showed statistically significant ($P<0.01$) increase of protease activity. In experimental conditions the tissues have shown increased protease activity in liver (33.20%) followed by kidney (32.19%), heart (28.83%) and muscle (26.83%) in multiple doses. The maximum increase was observed in multiple doses followed by double and single dose chlorpyrifos treated rats.

The results of glutamate dehydrogenase activity in the control and experimental albino rats under the study are given in Table-2. The experimental rats exposed to chlorpyrifos showed statistically significant ($P<0.01$) increase of glutamate dehydrogenase activity. The increase in glutamate dehydrogenase activity was dose and time dependent manner in chlorpyrifos treated rats. In experimental conditions the tissues have shown

increased glutamate dehydrogenase activity in liver (40.96%) followed by muscle (39.70%), heart (31.16%) and kidney (25.66%) in multiple doses. The maximum increase was observed in multiple doses followed by double and single dose chlorpyrifos treated rats.

Table 1: Changes in protease activity (µ moles of tyrosine/mg protein/hr) levels in different tissues of control and chlorpyrifos treated albino rats. Values in parentheses indicate percent change over control.

Name of the tissue	Control	Single Dose	Double Dose	Multiple Dose
Liver				
Mean	1.252±0.549	1.292	1.486	1.667
SD		±0.051	±0.060	±0.0573
PC		(3.218)	(18.688)	(33.208)
Kidney				
Mean	0.343±0.043	0.379	0.414	0.454
SD		±0.052	±0.054	±0.044
PC		(10.325)	(20.68)	(32.198)
Heart				
Mean	0.414±0.044	0.450	0.482	0.533
SD		±0.034	±0.056	±0.043
PC		(8.79)	(16.421)	(28.833)
Muscle				
Mean	0.529±0.0611	0.553	0.600	0.671
SD		±0.050	±0.050	±0.050
PC		(4.475)	(13.425)	(26.83)

All the values are mean ± SD of six individual observations.
SD – Standard Deviation.
PC – Percent change over control.

Table2: Changes in glutamate dehydrogenase (µ moles of formazon formed/mg protein/hr) levels in different tissues of control and chlorpyrifos treated albino rats. Values in parentheses indicate percent change over control.

Name of the tissue	Control	Single Dose	Double Dose	Multiple Dose
Liver				
Mean	0.415	0.462	0.520	0.585
SD	±0.010	±0.010	±0.006	±0.006
PC		(11.32)	(25.30)	(40.96)
Kidney				
Mean	0.208	0.222	0.236	0.260
SD	±0.073	±0.046	±0.082	±0.062
PC		(8.11)	(15.44)	(25.66)
Heart				
Mean	0.115	0.126	0.131	0.146
SD	±0.006	±0.003	±0.005	±0.003
PC		(10.06)	(16.84)	(31.16)
Muscle				
Mean	0.205	0.236	0.274	0.304
SD	±0.061	±0.083	±0.019	±0.014
PC		(11.85)	(25.57)	(39.70)

All the values are mean ± SD of six individual observations.
SD – Standard Deviation.
PC – Percent change over control.

Discussion

Under proteolysis, enhanced breakdown dominates over synthesis. While in the case of anabolic process, increased synthesis dominates the protein breakdown[16]. Increase in protease activity observed at single, double and multiple doses of chlorpyrifos on different tissues of albino rats were clearly reflected in breakdown of proteins.

Proteases were found to be activated during stress condition indicating a possible relation between inactivation of oxidative enzymes, reduction in energy production and acceleration of proteolysis[20]. Chlorpyrifos caused significant increases in protease activity in the treated rats; similarly, several authors reported increased protease activity in different animal models under pesticidal toxicity, such as in fishes treated with atrazine[21], treated with cypermethrin[22,23], in mice treated azadirachtin and monocrotophos[24], in rats treated with cypermethrin[25] and acephate[26]. Increased protease activity in tissues of *Tilapia mossambica* exposed to sodium selenite[27], in the tissues of mice exposed to aluminum acetate[28], in liver tissue of albino rat exposed to hexachlorophene[29]. The elevated protease activity, in general, indicates profound loss of proteins causing structural disorganization and disassembly of structural proteins in different tissues during chlorpyrifos toxicity.

GDH catalyzes the reversible reaction of oxidative deamination of glutamate to α-ketoglutarate and ammonia and plays an important role in the catabolism and biosynthesis of amino acid[16]. Glutamate Dehydrogenase occurs with high activity in the mitochondrial matrix it is commonly used as a marker for matrix space[30]. It has a great importance in neurotransmitter balance in brain tissue and maintenance of nitrogen in liver tissue. As GDH plays an important role in detoxification of ammonia[31], increased glutamate dehydrogenase activity was observed in the tissues of albino rat exposed to chlorpyrifos in the present investigation.

Glutamate dehydrogenase (GDH) is also known to play a crucial role in protein metabolism in the cells affected by a variety of effectors[32]. This enzyme has several metabolic functions with great physiological significance. 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 into urea in the liver. In the present study increase in GDH activity favors trans-deamination of amino acids to incorporate them into TCA cycle as keto acids. Therefore, a progressive elevation in the enzyme activity is noticed.

The elevation in GDH activity under toxic stress was also reported by some workers[, 34, and 35]. Begum[35] reported enhanced GDH activity in muscle and kidney tissues of *Clarias batrachus* for 10 days of cypermethrin toxicity, which indicates increased deamination of glutamate and formation of ammonia. Stimulated GDH activity under cypermethrin stress suggests the need for α-ketoglutarate in the TCA cycle for the liberation of energy. Increased GDH activity in liver tissues were observed in albino rats under sodium arsenate toxicity[36].

Changes in permeability properties of mitochondria and lysosomal damage are also known to elevate GDH activity[37]. Chlorpyrifos caused significant increases in GDH activity in the treated rats; similarly, several authors reported increased GDH activity in different animal models under pesticidal toxicity, such as in fishes treated with atrazine[21], in fishes treated with cypermethrin[38, 39]. Increase GDH activities observed under ammonium toxicity in albino rats[40].

The GDH activity was found to be elevated in all the tissues of chlorpyrifos treated rats. The elevated GDH activity levels indicate its contribution to ammonia production and glutamate oxidation during chlorpyrifos toxicity. The elevated free amino acid levels and their subsequent transamination towards the formation of glutamate leads to the consequent oxidative deamination reaction through GDH and also helps in supplying keto acids to TCA cycle for energy production.

Conclusion

It is observed that in the present study an organo phosphorus pesticide chlorpyrifos influences on protein metabolism in the liver, kidney, Heart and muscle of albino rats. The elevated protease activity, in general, indicates profound loss of proteins causing structural disorganization and disassembly of structural proteins in different tissues during chlorpyrifos toxicity. Enhanced activity of GDH indicates increased deamination of glutamate and formation of ammonia, stimulated GDH activity under chlorpyrifos stress suggests the need for α-ketoglutarate in the TCA cycle for the liberation of energy.

References

1. Torstensson, L, L. N Lundgren, J Stenstrom." Influence of climate and edaphic factors on persistence of glyphosate and 2,4-D in forest soils". *Ecotox. Environ. Safe*. 18. 1989:230-239.
2. Williams Eagle. "Persistence of dichlobenil in a sandy soil and effects on plant Growth". Weed Research. 19. 1979: 315- 319.
3. Santhakumar M, and M Balaji. "Acute toxicity on organophosphorus insecticide monocrotophos and its effects on behavior of an air-breathing fish, Anabas testudineus (Bloch)". J. Env. Biol. 21(2). 2000: 121–123.
4. Metcalf R. L. "The impact of the development of organophosphorus insecticides upon basic and applied sciences". *Bull. Entomol. Soc. Am.,* 5. 1959: 3-15.
5. Pryde L. T. "Environmental Chemistry, An Introduction". Cumming Publishing Co. Menlopark, San Diego, California. 1973.
6. BookHout C. C, and R. J Monroe. "Physiological Responses of Marine Biota to Pollutants. Academic Press, New York. 1977: 3.
7. Barrett A. J. "Introduction to the history and classification of tissue proteinases. In Proteinases in Mammalian Cell Tissues" Edited by A. J. Barrett. New York: North Holland.1977: pp. 1-55.
8. Ali S. Y and C. H Lack. "Studies on the tissue activities and proteolytic activity in the subcellular fractions of rabbit kidney". *Biochem.J.,* 96. 1965: 63.
9. Davies DTP, K Krakauer and G Weissman. "Neutral protease of granulocyte lysosomes: Inhibition and activation". *Fed. Proc.,* 29(1) 1970:784.
10. Stagni, N and B Debernard. Lysosomal enzyme activity in rat and beef skeletal muscle. *Biochem. Biophys. Acta.,* 170(1). 1968: 129-139.

11. Noguchi T and M Kandatsu. 'Some properties of alkaline protease in rat muscle with that in peritoneal activity cells". *Agri. Biol. Chem.,* 40. 1976: 927.

12. Pellegrino C and C Franzini. "An electron microscopic study of denervation atrophy in red and white skeletal muscle fibers". *Cell Biol.,* 17. 1963: 327.

13. Goldberg A. L and J. F Dice. "Intra cellular protein degradation in mammalian and bacterial cells" *Annu. Rev. Biochemistry,* 43. 1974: 835-869.

14. Venkata Swamy K. "Neurochemical studies during the development of behavioral tolerance to organophosphate toxicity in albino rats. Ph.D. Thesis, Sri Venkateswara University, Tirupati, India. 1991.

15. Knox W. E and O Greengard. "The regulation of some enzymes of nitrogen metabolism on introduction to enzyme physiology". In: Advances in enzyme regulation., 73: (Eds). G. Weber, Bergman Press, New York. 1965. 247.

16. Murray Robert K, K Daryl Granner a Peter Mayes and W Victor Rodwell. "In: Harper's Illustrated Biochemistry" International 26th Edition, The McGraw-Hill Companies, Inc. 2007: pp 46, 47.

17. Finney D. J. "Probit analysis" III Edition, Cambridge Univ. press, London. 1971. p.20.

18. Moore S and WH Stein. "Modified ninhydrin reagent for the photometric determination of amino acids and related compounds". *Journal of Biological Chemistry,* 221. 1954: 907-913.

19. Lee Y L and A. A Lardy. "Influence of thyroid hormones on L-glycerophosphate dehydrogenases in various organs of the rat". *Journal of Biological Chemistry,* 240. 1965: 1427-1430.

20. Henderson S. A, A. L Black and G. A Brooks. "Leucine turnover and oxidation in trained rats during exercise". *American. Journal of Physiology*, 249. 1985: E137 – E144.

21. Prasad T. A, T Srinivas, and D. C Reddy. "Modulations in nitrogen metabolism in the hepatic and neuronal tissues of fish, *Tilapia mossambica* exposed to atrazine". *Biochem. Int.,* 23(2). 1991: 271-9.

22. David M, S. B Mushigeri, R Shivakumar and G. H Philip. "Response of *Cyprinus carpio* (Linn) to sublethal concentration of cypermethrin: alterations in protein metabolic profiles". *Chemosphere,* 56. 2004: 347-352.

23. Prasanth M. S and M David. "Changes in nitrogen metabolism of the freshwater. Fish *Cirrhinus mrigala* following exposure to cypermethrin". *J. Basic Clin. Physiol. Pharmacol.,* 17 (1). 2006: 63-70.

24. Sivaiah, U. "Azadirachitin and monocrotophos effect on haematoloical, teratological, bio chemical and cytoarchitectural studies in albino mice". Ph.D., Thesis, S.V. University, Tirupati, A.P., India.2006.

25. Nagarjuna A. "Effect of cypermethrin on hematological, protein metabolism and histological studies in albino rats". Ph.D. Thesis, Sri Venkateswara University, Tirupati, India. 2007.

26. Rajeswari ISR. "Toxicity of organophosphate Compound acephate O. N haematological, selected biochemical parameters and histological studies in wistar strain rats". Ph.D. Thesis, Sri Venkateswara University, Tirupati, India. 2008.

27. Samson Raju C, P Jacob Doss, T Venkatesulu, A Nagarjuna and K Jayantha Rao. "Effect of sodium selenite on protein metabolism in a fish *Tilapia mossambica". Aquacult,* 7(1). 2006:21-25.

28. John Sushma N, U Sivaiah, P Jacob Doss and K Jayantha Rao. "Impact of aluminum acetate on protein metabolism of albino mice". *Indian Journal of Comparative Animal Physiology,* 24(1). 2006: 66-71.

29. Suhasini N, V Lokanatha, C. P Sahitya and W Rajendra. "Alterations in the protein catabolism and transamination pattern in the rat liver on repeated hexachlorophene treatment". *Toxicology International,* 13(1). 2006: 33-38.

30. Kovacevic Z and J. D Mc Givan. ": itochondrial metabolism of glutamine and glutamate and its physiological significance". Physiol. Rev., 63(2). 1983:547-605.

31. Campbell JW. "Nitrogen excretion in: Comparative animal physiology' (Ed Prosser. CI) Saunders Co., London. 1973: 279-316.

32. Ramanadikshitulu A. V, K Narayana Reddy and K. S Swamy." Effect of selected metal ions on glutamate dehydrogenase activity in cell free extract of goat liver". *Indian Journal of Experimental Biology,* 14. 1976: 621-623.

33. Radhakrishnaiah K, A Suresh, B Urmila Devi and B Sivaramakrishnaiah. "Effect of mercury on the lipid metabolic profiles in the organs of *Cyprinus carpio* (Linnaeus)". *J. Mendel.,* 8. 1991: 123-125.

34. Sreedevi P, A Suresh, B Siva Ramakrishna, B Prabhavathi and Radhakrishnaiah. "Bioaccumulation of nickel in the organs of fresh water fish *Cyprinus carpio* and fresh waters mussel *Lamellidens marginalis". Chemosphere,* 24. 1992: 29-36.

35. Begum G. "Cypermethrin-induced biochemical perturbations in fresh water fish Clarias batrachus at sublethal exposure and after released into fresh water". Drug and Chemical Toxicology, 30. 2007:55-65.

36. Devaraju T, K Sujatha, S Madhava Rao and K Jayantha Rao. "Impact of sodium arsenate on selected enzymes and Histopathological studies in albino mice". International Journal of Pharma and Bio Sciences. 1 (3). 2010: Jul-Sep,1-7.

37. Johnson B.E and D Farrington. "Lysosomes and the reaction of skin to ultraviolet radiation". *Journal of Invertebrate Dermatology,* 53(2). 1969: 85-94.

38. Khalid Abdullah Al-Ghanim. "Effect of a Synthetic Pyrethyroid, Cypermethrin, on Aminotransferases and Glutamate Dehydrogenase Activities in Gill, Liver and Muscles of a Freshwater Fish, Cyprinus carpio". Pakistan J. Zool., 46(4). 2014: 997-1001.

39. Wasim Yhasmine. "Cypermethrin Toxic effect on enzyme activities in freshwater fish (Cyprinus carpio)". Advances in Aquaculture and Fisheries Management. 1(9). 2013: 094-097.

40. Sireesha A and P Neeraja. "Induced ammonia stress on development of albino rat through study of certain biochemical components". Int. J. Adv. Biol. Research, 2(4). 2012: 704-707.

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