Research Article **Biochemistry**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

TOXIC IMPACT OF LETHAL AND SUBLETHAL CONCENTRATIONS OF PHORATE ON THE TOTAL PROTEIN LEVELS IN DIFFERENT TISSUES OF COMMON CARP *Cyprinus carpio*

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ABSTRACT

Fingerlings of *Cyprinus carpio* were exposed to acute lethal toxicity of Phorate for one day and 4 days and chronic sublethal toxicity of Phorate for 1, 7, 15 and 30 days. After the completion of stipulated exposure period the total protein levels were estimated in the target organs like gill, liver, muscle, kidney and brain of the fish. Significant alterations were observed in the levels of total protein content in all the organs of the fish exposed to both acute lethal and chronic sublethal toxicity of Phorate. The alterations in the levels of total protein content were significantly dose dependent.

KEY WORDS: **Cyprinus carpio, acute lethal, Phorate, chronic sublethal.**

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INTRODUCTION

Water is one of the most essential needs for the survival of life on earth. Water covers 71% of the earth's surface [1] and is vital for all forms of life. The aquatic environment is currently under threat by the indiscriminate use of synthetic pesticides by the human activities and causing high risk to non-target organisms [2] including fish. Pesticides used for controlling pests in agriculture are one of the major causes of aquatic pollution. Heavy dependence modern agriculture on agrochemicals such as pesticides is emerging as a threat to the ecological balance of aquatic ecosystems. Pesticides are carried into the aquatic ecosystems by surface runoff from sites of application and therefore the health of aquatic ecosystem is being adversely affected because they serve as an ultimate sink for these pesticides [3]. These pesticides are also found to be highly toxic not only to fish but also to other organisms which constitute food of the fish.

Among synthetic pesticides organophosphates are widely used in agriculture and in health and hygiene programs due to their high effectiveness as insecticides but less persistence in the environment. The shift from organochlorines to organophosphates has resulted into increased occurrence of organophosphates into water bodies causing acute and chronic toxicity to fish fauna[4,5, 6]. Phorate is an organophosphorus insecticide and acaricide used to control sucking and chewing insects, leafhoppers, leafminers, mites, some nematodes, and rootworms [7, 8]. It is used in pine forests and on root and field crops including corn, cotton, coffee, some ornamental and herbaceous plants and bulbs. According to United States Environmental Protection Agency, Phorate belongs to Toxicity Class I. Pesticides of this class are most toxic, requires Signal Word: "Danger-Poison", with skull and crossbones symbol.

Proteins being involved in the architecture and physiology of the cell, they seem to occupy a key role in cell metabolism [9]. Umininger [10] suggested that carbohydrates represent the principal immediate energy precursors for fishes exposed to stress, while proteins are the energy sources during chronic periods of stress. The amino acids formed by protein degradation, on one hand, serve for the synthesis of required proteins and, on the other hand, useful for meeting the energy demands [11].

There is an increased evidence of pesticide protein interaction, which is relevant to the mode of action of insecticide [12]. Protein, the chief organic macromolecule for all aspects of cellular structure and function, is expected to react first upon pesticide exposure. Pesticides alter protein synthesis by proteolysis or protein hydrolysis [13, 14, 15, 16, 17]. Pesticides impair protein metabolism leading perhaps into disarray of functional and structural status of the cell [18].

The present study is aimed to assess the changes in the total protein levels in the selected organs like gill, liver, muscle, kidney and brain of the edible fresh water teleost fish, *Cyprinus carpio* exposed to acute lethal and chronic sub-lethal toxicity of phorate.

MATERIALS AND METHODS

Animal Selected

The Indian major carp *Cyprinus carpio* (Linnaeus, 1758) has been selected as test species for the present investigation. It is an economically important edible fresh water fish, having great commercial value. Fish were collected from the department of fisheries, Anantapur, Andhra Pradesh and were transferred into large cement tanks with sufficient dechlorinated tap water and allowed to acclimatize for 15 days. The animals were starved for 24 hours prior to each estimation to avoid any influence of differential feeding.

Test Chemical

Pesticide selected for this study is phorate (O,O-diethyl S-ethylthiomethyl phosphorodithioate), an organophosphorus

insecticide which is widely used throughout the world and also in India and Andhra Pradesh as a broad-spectrum insecticide on numerous crops. Commercial names of phorate are Thimet, Rampart, Granutox Agrimet etc. and its molecular formula is $C_7 H_{17} O_2 PS_3$.

Acute and Chronic toxicity procedures

Lethal concentration (LC_{50}) of phorate to *Cyprinus carpio* was determined by Probit method of Finney [19]. LC_{50}/96 hours (0.71 ppm/l) of phorate was taken as lethal concentration to study the acute toxicity and one-tenth of the LC_{50}/96 hours (0.071 ppm/l) concentration of phorate was taken as the sub-lethal concentration in chronic toxicity study.

Experimental Design

160 fishes were divided into two batches, again batch I was divided into 3 groups and batch II was divided into 5 groups comprising of 20 fishes each. Batch I was exposed for acute toxicity of Phorate (exposed to lethal concentrations (LC_{50}) of Phorate = 0.71 ppm/lit) and batch II was exposed for Chronic toxicity of Phorate (exposed to sub lethal concentration = $1/10^{th}$ of LC_{50} - 0.071 ppm/lit). In batch I, group 1 was considered as normal control, group 2 and 3 were experimental groups. The fishes of group 2 were exposed for one day and group 3 for 4 days. In batch II, group 1 was considered as a normal control group, group 2, 3, 4 and 5 were experimental groups. The fishes of group 2 were exposed for one day, group 3 for 7 days, group 4 for 15 days and group 5 for 30 days.

Analysis of Total Protein content levels

At the end of exposure period, the healthy fishes were taken out and were stunned to death and the target organs like gills, liver, muscle, kidney and brain were dissected out from each animal using sterilized instruments. The total proteins were estimated using Folin phenol reagent method as described by Lowry et al [20]. The total protein content analysis was carried out in the organs from six animals at each exposure period and the mean of six is taken into consideration. Similar studies were made on the animals from normal medium served as controls.

Statistical analysis

DMR (Duncan's Multiple Range) test had been employed for the statistical analysis of the total protein levels data. P value (level of significance) is significant at < 0.05.

RESULTS AND DISCUSSION

The data on the levels of total proteins (mg/gm wet wt.) in the organs such as gill, liver, muscle, kidney and brain of the fish *cyprinus carpio* at one and 4 days on exposure to acute toxicity of phorate and 1, 7, 15 and 30 days on exposure to chronic toxicity of phorate, besides controls, are presented in the Table-I and Graph-I. Further for comparative assessment, the differences obtained in relation to controls of each organ at the above said exposure periods in acute and chronic toxicity study of phorate, were converted into percentages of the corresponding controls and those percent values are also given in the respective table and was plotted a graph of percent changes against exposure periods in Graph-I. From the data presented in the Table-I and Graph-I it is seen that, relative to controls, the levels of total protein content in all the organs of the fish exposed to phorate decreased significantly ($P<0.05$) at one and 4 days of exposure in acute toxicity in the order of 1>4 and at one and 7 days of exposure in chronic toxicity followed by a gradual increase of them at day 15 and day 30 significantly ($P<0.05$) in the fish exposed to chronic toxicity of phorate in the order of 1>7<15<30.

In the present study a decrease in total protein content in the gill, liver, muscle, kidney and brain of the fish *cyprinus carpio* at one and 4 days of exposure in acute toxicity of phorate and at one and 7 days of exposure in chronic toxicity of phorate indicates the initiation of the breakdown of proteins under toxic stress. Decrease in total protein content suggests its metabolic utilization under phorate toxic stress. The degree of

proteolysis gradually enhanced in the organs of the fish from day 1 to day 4 in acute toxicity of phorate and from day 1 to day 7 in chronic toxicity of phorate. This indicates that the breakdown of proteins may dominate over synthesis under enhanced proteolytic activity [21]. Several authors reported decreased total protein contents in different animal models under pesticidal toxicity, such as in fishes treated with atrazine [22], carbofuran [23], cypermethrin [24, 25, 26, 27, 28], fenvalerate [29], quinalphos [30], chlorpyrifos [31] and cockerels treated with imidacloprid and quinalphos [32].

The reason for the decrement of proteins is that tissue protein might be metabolized to produce glucose by the process of gluconeogenesis and glucose is utilized for energy production during stress condition by pesticide [33] and this may be the cause for lower amounts of protein levels in experimental animal tissues. The reduction in protein content in animals treated with pesticides could be attributed to changes in protein and free amino acid metabolism and their synthesis in liver [34].

Table-I

Total protein content (mg/gm wet wt) in different organs of the fish Cyprinus carpio at different periods of exposure to acute and chronic toxicity of phorate (n=6).

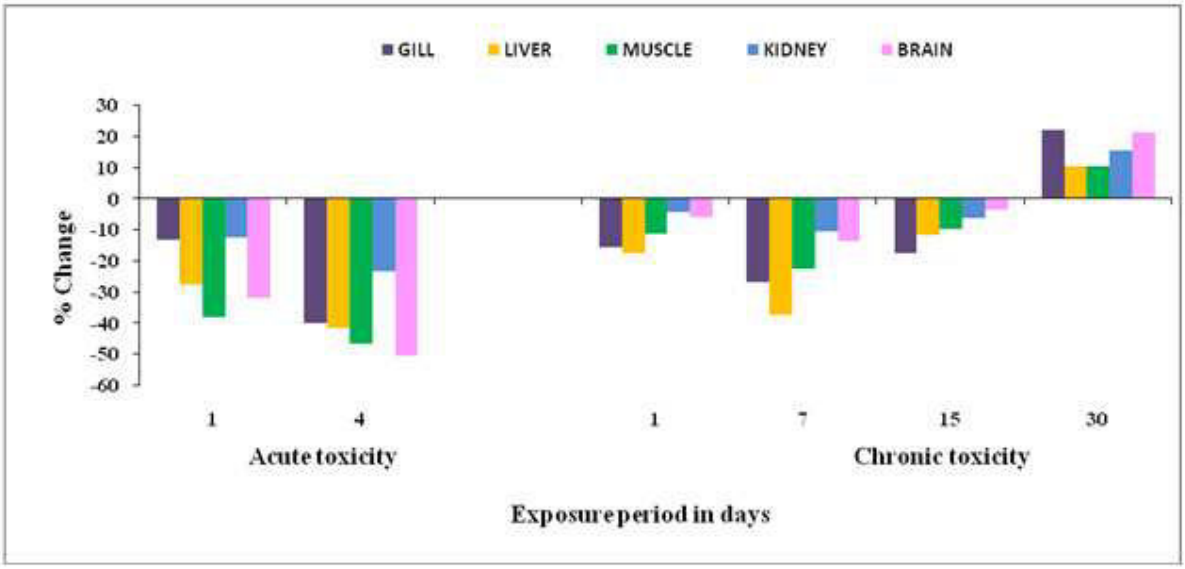
ORGAN		EXPOSURE PERIOD IN DAYS							
		ACUTE TOXICITY			CHRONIC TOXICITY				
		CONTROL	1	4	CONTROL	1	7	15	30
GILL	Mean S.D. ± % change	66.038c 0.312	57.218b 0.213 -13.35	39.561a 0.306 -40.09	66.038d 0.312	55.676c 0.048 -15.69	48.141a 0.113 -27.10	54.321b 0.053 -17.74	80.586e 0.058 +22.03
LIVER	Mean S.D. ± % change	141.336c 0.436	101.956b 0.524 -27.86	82.355a 0.306 -41.73	141.336d 0.436	116.026b 0.089 -17.90	88.110a 0.063 -37.65	124.328c 0.050 -12.03	155.853e 0.0835 +10.27
MUSCLE	Mean S.D. ± % change	106.268c 0.4807	65.628b 0.367 -38.24	56.465a 0.340 -46.86	106.268d 0.4807	94.090b 0.103 -11.45	82.135a 0.103 -22.70	95.643c 0.075 -9.99	117.288e 0.086 +10.36
KIDNEY	Mean S.D. ± % change	90.343c 0.405	78.935b 0.235 -12.62	69.165a 0.188 -23.44	90.343d 0.405	86.271c 0.052 -4.50	80.590a 0.061 -10.79	84.648b 0.067 -6.30	103.983e 0.157 +15.09
BRAIN	Mean S.D. ± % change	97.620c 0.519	66.370b 0.438 -32.01	48.250a 0.398 -50.57	97.620d 0.519	91.863b 0.151 -5.89	84.101a 0.274 -13.84	93.985c 0.098 -3.72	118.283e 0.152 +21.16

Values with different superscripts with in the column are significantly different from each other at P<0.05 according to Duncan's Multiple Range Test (DMR) test. The values below the mean are percent changes over the respective controls.

The gradual increase in total protein content in the organs of the fish at 15 and 30 days of exposure to chronic toxicity of phorate indicate the domination of protein synthesis over breakdown during chronic sublethal toxic stress. It also indicates the synthesis of proteins to develop resistance to the imposed toxic stress. This indicates that the synthesis of proteins may dominate over the breakdown under enhanced anabolic process [21]. It could be due to slow activation of detoxification mechanisms by increasing their protein output and turnover. The removal of pesticide from general intracellular environment helps the animal to adapt to toxic stress. On prolonged exposure of the fish for a period of 15 and 30 days to phorate, the animal could regain its protein synthetic potentials and reorganize the aminoacids by activating the transaminases. These changes indicate the ability of the fish to resist to subacute phorate toxic stress on a long period of exposure.

Graph-I

Total protein content (mg/gm wet wt) in different organs of the fish Cyprinus carpio at different periods of exposure to acute and chronic toxicity of phorate .



All the values are mean ± SD of six individual observations.

CONCLUSION

Thus in the present investigation the results obtained shows that this pesticide, phorate seems to exert significant effect on the levels of total protein content in different tissues at different exposure periods in acute and chronic toxicity in the fish *cyprinus carpio* by altering the protein metabolism. The alterations in the levels of total protein content were significantly dose dependent.

REFERENCES

1. CIA- The world fact book, Central Intelligence Agency (2008), www.cia.gov/ library/ publications/ the-world-factbook.
2. Kumar A, Prasad MR, Srivastava K, Tripathi S and A.K. Srivastava, Branchial Histopathological Study of catfish *Heteropneustes fossilis* following exposure to purified Neem extract, Azadirachtin. World journal of zoology, 5(4): 239-243, (2010).
3. Singh RN, Pandey PK, Singh NN and Dass VK, Acute toxicity and behavioral responses of common carp *Cyprinus carprio* (Linn.) to an Organophosphate (Dimethoate). World Journal of Zoology, 5(3):183-188, (2010).
4. Rao JV, Begum M, Pallela M, Usman PK and Rao RN, Chnages in behavior and brain acctylcholinestarase activity in mosquito fish, *Gambusia affinis* in relation to sublethal exposure of chlorphyrifos. Intl. j. Environ Res. Public health, 2(3-4): 478-483, (2005).
5. Velmurugan B, Selvanayagam M, Cangiz E and Unlu E, The effects of monocrotophos to different tissues of freshwater fish *Cirrhnus mrigala.* Bull. Environ. Contam toxicol. 78(6): 450-454, (2007).
6. Singh R.N, Pandey RK, Singh NN and Das VK, Acute toxicity and behavioral responses of common carp *Cyprinus carpio* (Linn.) to an Organophosphate (Dimethoate). WJZ, 4: 70 -75, (2009).
7. Wagner SL, The acute health hazards of pesticides. In Chemistry, Biochemistry and Toxicology of Pesticides. Witt, J. M., Ed. Oregon State University Cooperative

Extension Service, Corvallis, OR, (1) 5-2, (1989).
8. Gallo MA and Lawryk NJ, Organic phosphorus pesticides. In Handbook of Pesticide Toxicology, Hayes WJ Jr and Laws ER Jr, Eds. Academic Press, New York, NY, 5-3, (1991).
9. Murray, Robert K, Daryl K, Granner, Peter A and Mayes et al, In: Harper's Illustrated Biochemistry. International 26th Edition, The McGraw-Hill Companies, pp46, 47, Inc (2007).
10. Umininger BL, Physiological studies on super cooled kill fish, *Fundulus heteroclitus*, III. Carbohydrate metabolism and survival at subzero temperature. J Exp Zool, 173: 159-174 (1970).
11. Krishnamohan Reddy B, Metabolic modulation of fatigue with special reference to lactate and ammonia metabolism in different skeletal muscle fibre types of albino rats. Ph.D. Thesis, Sri Venkateswara University, Tirupati, India, (1986).
12. Casida J.E, Gammon DW, Glickman AH and Lawrence L.J, Mechanisms of selective action of pyrethroid insecticides. Ann. Rev. Pharmacol. Toxicology, 23: 413-438, (1983).
13. 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).
14. Madhava Rao S, Biodynamics of *Rana hexadactyla* under azadirachtin impact (with special reference to selected biochemical and electron microscopy studies). Ph.D. Thesis, Sri Venkateswara University, Tirupati, India, (2007).
15. Begum G, Cypermethrin – induced biochemical perturbations in freshwater fish *Clarias batrachus* at sublethal exposure and after released into freshwater. Drug and Chemical Toxicology, 30:55-65, (2007).
16. Nagarjuna, Effect of cypermethrin on hematological, protein metabolism and histological studies in albino rats. Ph.D. Thesis, Sri Venkateswara University, Tirupati, India, (2007).
17. Rajeswari ISR, Toxicity of organophosphate Compound acephate on haematological, selected biochemical parameters and histological studies in wistar strain rats. Ph.D. Thesis, Sri Venkateswara University, Tirupati, India, (2008).
18. Shukla OP, Omkar and A.K. Kulshrestha, In: Pesticides, Man and Biosphere. First Edition, APH Publishing Corporation, New Delhi, (1998).
19. Finney DJ, Probit Analysis, 3rd edition, Cambridge University press, Cambridge: pp.333 (1971).
20. Lowry OH, Rosenbrough NJ, Farr AL and Randall RS, Protein measurement with folin-phenol reagent. Journal of Biological Chemistry, 193: 265-273, (1951).
21. Harper HA, Rodwell VM and Mayer PA, In: Review of Physiological Chemistry, 17th edition, Longe Medical Publications, Maruzer Company Limited, California (1979).
22. Prasad TA, Srinivas T and Reddy DC, Modulations in nitrogen metabolism in the hepatic and neuronal tissues of fish, *Tilapia mossambica* exposed to atrazine. Biochem Int, 23(2):271-279 (1991).
23. Begum G, Carbofuran insecticide induced biochemical alterations in liver and muscle tissues of the fish *Clarias batrachus* (linn) and recovery response. Aquatic Toxicology, 66: 83-92, (2004)
24. Philip GH and Rajasree BH, Action of cypermethrin on tissue transamination during nitrogen metabolism in *Cyprinus carpio*. Ecotoxicology and Environmental Safety, 34: 174-179, (1996).
25. Atamanalp M., Keles M.S, Haliloglu HI and Aras M.S, The effects of cypermethrin (a synthetic pyrethroid) on some biochemical parameters (Ca, P, Na and TP) of rainbow trout *Oncorhynchus mykiss*. Turkish Journal of Veterinary Animal Sciences, 26: 1157-1160, (2002).
26. Das BK and Mukherjee SC, Toxicity of cypermethrin in *Labeo rohita* fingerlings: biochemical, enzymatic and hematological consequences. Comparative Biochemistry and Physiology Part C, 134: 109-121, (2003).
27. David M., Mushigeri SB, Shivakumar R and Philip GH, Response of *Cyprinus carpio* (Linn) to sublethal concentration of

cypermethrin: alterations in protein metabolic profiles. Chemosphere, 56: 347-352, (2004).
28. Begum G, Cypermethrin – induced biochemical perturbations in freshwater fish *Clarias batrachus* at sublethal exposure and after released into freshwater. Drug and Chemical Toxicology, 30: 55-65, (2007).
29. Tripathi G and Verma P, Fenvalerate induced changes in a cat fish, *Clarias batrachus*: metabolic enzymes, RNA and protein. Comparative Biochemistry and Physiology, 138: 75-79, (2004).
30. Das BK and Mukherjee SC, Chronic toxic effects of quinalphos on some biochemical parameters in *Labeo rohita* (Ham.). Toxicology Letters, 114(1-3): 11-8, (2000).
31. Firdous Ahmad Malla, Assessment of total protein concentration in liver of fresh water fish, *Channa punctuates* (bloch.) with special reference to an organophosphate insecticide, chlorpyrifos. Int J Pharm Bio Sci, 3 (2): (B) 567 – 571 (2012).
32. Siddiqui MA, Toxicological and Immunological studies of subacute exposure of cockerels to imidacloprid and quinalphos. M.V.Sc. Thesis, Gujarat Agricultural University, Anand, India, (2004).
33. Jayantha Rao K, Effect of a Systemic Pesticide Phosphomidon on some aspects of metabolism in fresh water fish. *Tilapia mossambica* (peters). Ph.D., Thesis, S.V. University, Tirupati, A.P., India, (1982).
34. Rivarola VA and Balegno HF, Effect of 2, 4-dichloropheneoxyacetic acid on polyamine synthesis Chinese hamster ovary cells. Toxicology Letters, 56: 151-157, (1991).