ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 5, Issue, 01, pp.322-324, January, 2013

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

IDENTIFICATION AND SPECIFICATION OF STRESS PROTEINS IN THE BRAIN TISSUE OF *Carassius auratus* Exposed TO Chlorpyrifos

***Vaidehi, J., Ronald Ross, P., Paramanandham, J., Yogalakshmi, K. and Ramakotti, P.**

Department of Zoology, Faculty of Science, Annamalai University, Annamalai Nagar- 608 002

ARTICLE INFO

Article History:
Received 20th October, 2012
Received in revised form
14th November, 2012
Accepted 17th December, 2012
Published online 15th January, 2013

Key words:

Hazardous, Translocated,
Organophosphorus,
Polypeptides, Transcription.

ABSTRACT

Environmental pollutants pose a huge menace to all living organisms either directly or indirectly. Pesticide contaminations in the aquatic ecosystem affect the fishes at large leading to severe physiological and morphological stress response. Fishes in turn to counter the stress factors, ought to synthesize a group of novel proteins called stress proteins which play an important role in the stress-resistance of cells against the toxic chemicals. In the present study, the ornamental fish *Carassius auratus (var.,) auratus* was exposed to different concentrations of the organophosphorous pesticide, chlorpyrifos in various exposure periods and the type of stress proteins synthesized were investigated.

INTRODUCTION

Over exploitation of natural resources lead to the release of pollutants to the environment. Insecticides, plastics, heavy metals, toxic trace elements and hazardous wastes which are persistent still worsen the problem. The harmful and poisonous chemicals persistent in the environment cause severe damage to the living beings and the ecosystem. The pesticidal stress in fishes shows disorder in the form of behavioral changes through the production of shock proteins (Osman *et al*., 2010). One of the most prevalent cellular responses to stress is the synthesis of new classes of proteins (Iwama *et al*., 2004; Multhoff, 2007; Keller *et al*., 2008). These proteins help to protect the cell during stress condition and later quicken recovery. All organisms respond to environmental stress, such as heat, pollutants or similar other stresses, by a rapid, vigorous and transient acceleration in the rate of synthesis of a group of proteins collectively called the Heat Shock Proteins (HSPs) or more generally stress proteins (Airaksinen *et al*., 2003; Hollander *et al*., 2004; Mao *et al*., 2005). The syntheses of such proteins are strongly stimulated following perturbations of the cellular environment (Morimoto *et al*., 1990). These proteins appear to play a role in the cellular resistance to stress (Landry *et al*., 1989, Mehlen *et al*., 1993; Heinz *et al*., 2012). The stress proteins are known to help cells under stress by maintaining the proteins in the correct conformation by aiding the proteins to be properly translocated to different organelles in the cell (Craig *et al*., 1994). But scanty information is available on stress protein production towards pesticide stress. Hence in the present study the impact of an organo phosphorus pesticide chlorpyrifos on the production of stress proteins in the freshwater fish *Carassius auratus* was found out.

MATERIALS AND METHODS

Experimental fish

The gold fish *Carassius auratus (var.,) auratus* is a freshwater fish in the family Cyprinidae of order Cypriniformes. It was one of the earliest fish to be domesticated, and is one of the most commonly kept aquarium fish.

Experimental pesticide

Chlorpyrifos (IUPAC name: *O, O-diethyl O-3,5,6- trichloropyrin-2yl phosphorothioate*) is an organophosphate insecticide. Chronic exposure of chlorpyrifos to animals leads to neurological effects, developmental disorder, and autoimmune disorders.

Collection and Maintenance

The fishes were collected from an aquarium at Chidambaram Town. From the collection, fishes of 25 to 30 g were chosen and reared separately in plastic troughs to get them acclimatized in the laboratory condition. The oxygen content in the water was maintained throughout the experiment by an aerator, so that oxygen was not acting as a limiting factor. The fishes were fed with pelletized meal containing groundnut oil cake, Bengal gram powder and rice bran. The fishes were acclimatized to laboratory conditions such as feeding and temperature for a period of 15 days prior to the initiation of the experiment.

Determination of Lethal Concentration (LC_{50})

In the present study, an attempt was made to study the formation of stress protein when the animals were exposed to chlorpyrifos. Primarily the LC50 of chlorpyrifos was fixed. The percentage of mortality for a period of 96hrs was observed and the data is incorporated in the Table given under. 50% mortality for a period of 96 hrs was observed in the concentration of 0.2 ml/l. Therefore the LC_{50} of chlorpyrifos for this particular species was fixed as 0.2 ml/l. (Finney, 1964)

Experimental Design

To study the sublethal doses of chlorpyrifos on the changing protein profile in freshwater fish *Carassius auratus*, fishes weighing about 25 to 30g were grouped into 3, representing 3 different pesticide

Corresponding author: Vaidehi, J., Department of Zoology, Faculty of Science, Annamalai University, Annamalai Nagar- 608 002.

concentrations such as 0 ml/l (control), 0.025 ml/l and 0.1 ml/l as the 1/8 and 1/2 of the lethal concentration 0.2 ml/l. For the study of change in the tissue profile, fishes were exposed to acute shock towards the pesticide, chlorpyrifos for a period of 15 mins, 60 mins and 180 mins. After the stipulated time of pesticide exposure, fishes were sacrificed and the brain tissue was collected for the analysis of stress proteins using gel electrophoresis technique.

Table 1. Percentage mortality of *Carassius auratus* exposed to various concentrations of chlorpyrifos at different exposure periods

Conc. In (ml)	Mortality percentage (Hours)					Remarks
	12	24	48	72	96	
0.01	0	0	0	0	0	Non- lethal dose
0.05	0	0	0	0	0	Non- lethal dose
0.1	0	0	0	0	0	Non- lethal dose
0.2	0	25	0	0	50	LC_{50}/96hrs
0.25	0	0	50	0	75	
0.5	0	25	50	100	0	
1.0	10	0	50	0	0	

Electrophoresis technique

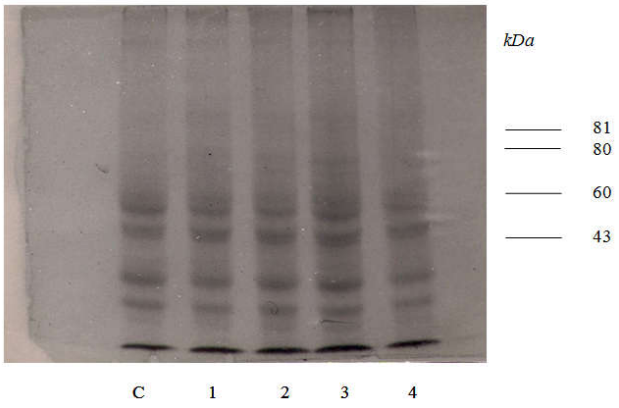
SDS polyacrylamide gel electrophoresis was carried out to study the protein profile in the brain tissue. PAGE has been extensively employed with denaturing agents like SDS and mercaptoethanol. SDS and mercaptoethanol break up a majority of macromolecular complex proteins into their rod-like linear polypeptides. These molecules were subjected to electrophoretic migration according to their molecular size and molecular weight.

RESULT

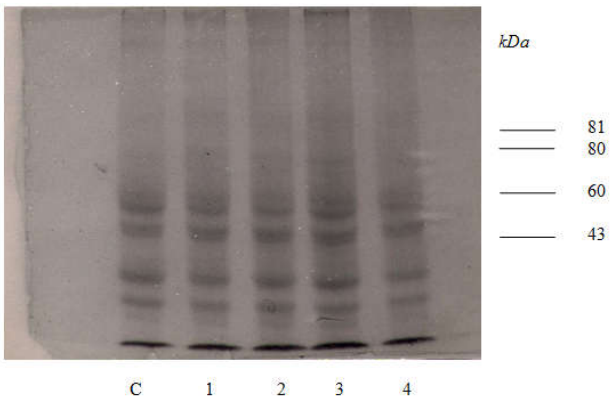
Brain tissue samples of chlorpyrifos treated *Carassius auratus* showed different protein profiles on electrophoretic analyses. The protein profile of pesticide treated brain tissues showed entirely different response (Plate 1). The kinds of proteins, exhibited alteration in their quantities are 206kDa, 37kDa, 22kDa and 13kDa. Among the four aforementioned fractions, the fractions such as 206kDa and 37kDa proteins are found disappearing in the pesticide treated groups from that of the control. Increased doses of chlorpyrifos from 0.025 ml/l to 0.1 ml/l to the fish brought marked changes in the protein profile of brain tissues (Plate 1 and 2). There are four different kinds of protein bands appearing new in the pesticide treated groups which are not seen in the control. The protein band with 81 kDa appeared in the 60 min treated as well as 180 min treated groups while the protein band with around 80 kDa appeared only in the 180 mins treated group. Similarly the protein fractions with 60 kDa and 43 kDa which were not found in control groups appeared increasing in its quantity with increasing duration of pesticide exposure.

DISCUSSION

The results showed the synthesis of new proteins and the intensification of still other polypeptides during pesticide stress and these proteins are known as stress proteins (Table 2).



Changes in the protein profile of the brain of *Carassius auratus* exposed to chlorpyrifos (0.025 ml/L) for a period of 15 and 60 mins.



Changes in the protein profile of the brain of *Carassius auratus* exposed to chlorpyrifos (0.1 ml/L) for a period of 60 and 180 mins.

This specific cellular stress response involves shutting off the general transcription and activating the transcription of selected set of genes coding for the stress proteins. These are involved in protecting animals from damage as a result of exposure to a wide variety of environmental stressors. The observed results indicate that stress protein synthesis is highly tissue specific. According to Brenda (1994) tissue specificity is probably a result of the differences in gene expression among specialized cell types and the extent of tissue damage. The protein fraction with 80 kDa is of unique in appearance in the pesticide induced fish. However, similar reports were made with salinity stress in *Cyprinus carpio* (Ranjini, 2001) and *Mystus vittatus* (Sujatha, 1997). The appearance of new low molecular weight proteins got intensified with the increasing time of exposure to chlorpyrifos. Similar observations were made by Theodarakis *et al*., (1992), Dyer *et al*., (1993); Sanders *et al*., (1995); Keller *et al*., (2008) and Eder *et al*., (2009). This study of stress response to environmental stressors may serve to understand the regulation of induction of stress proteins. It also implies the major role played by stress proteins in the cells undergoing stress due to the pesticides. Though several stress proteins are expressed during pesticide stress, it is of immense need to

Table 2. Proteins expressed in the brain tissue of *Carassius auratus* exposed to chlorpyrifos

Tissue tested	Protein MW (kDa)	Concentration of chlorpyrifos in ml/l (0.025)			Concentration of chlorpyrifos in ml/l (0.1)		
		Duration of exposure (mins)			Duration of exposure (mins)		
		0	15	60	0	60	180
Brain	206	√	–	–	–	–	–
	81	–	–	–	–	√	√
	80	–	–	–	–	–	√
	60	–	–	–	–	√	√
	43	–	–	–	–	√	√
	37	√	–	–	–	–	–
	22	√	√√	√√	–	–	–
	13	√	–	√	–	–	–

characterize the functions of each protein separately which may serve as a continuation of the present study and may be suggested for the possibility of using specific stress proteins as biomarkers for complex environmental pollution (Wang *et al*., 2007).

REFERENCES

Airaksinen, S., Rabergh, C.M.I., Lahti, A., Kaatrasalo, A., Sistonen, L., and Nikinmaa, M, 2003. Stressor dependent regulation of the heat shock response in zebrafish, *Danio rerio. Com. Biochem. and Physiol.,* 134: 839-846.

Brenda, S, 1994. Stress proteins as molecular chaperones: Implications for Toxicology, Environmental Health Perspectives. 102: 6-7.

Craig, E.A., Weissman, J.S., and Hormich, A.L, 1994. Heat Shock Proteins and molecular chaperones: Mediators of Proteins conformation and turnover in the cell. *Cell,* 78: 365-372.

Dyer, S.D., Dickson, K.L., and Zimmerman, E.G, 1993. A laboratory evaluation of the use of stress proteins in fish to detect changes in water quality. *Environmental toxicology and risk assessment*. 247-261. In: Landis., W.G., Hughes, J.S., and Lewis, M.A (*eds*), ASTEM STP1179. American Society for testing and materials Philapelphia, PA.

Eder, K.J., Leutenegger, C.M., Kohler, H.R., and Werner, I, 2009. Effects of neurotoxic insecticides on heat shock proteins and cytokine transcription in Chinook salmon (*Oncorhynchus tshawytscha*). *Ecotoxicol. and Env. Safety*, 72: 182-190.

Finney, D.J, 1964. Probit analysis: A statistical treatment of sigmoid Response curve. Cambridge University Press, U.K., 20-31.

Heinz, P., Marten, R.A., Linshy, V.N., Haap, T., Geslin, E., and Kohler, H.R, 2012. 70kD Stress protein (70Hsp) analysis in living shallow-water benthic foraminifera. *Marine Biol. Res.,* 8: 677-681.

Hollander, J.M., Martin, J.L., Belke, D.D., Scoth, B.T., Swanson, E., Krishnamoorthy, V., and Dillmann, W.H, 2004. Over expression of wild-type heat shock protein 27 and a nonphosphorylatable heat shock protein 27 mutant protects against ischemia/reperfusion injury in a transgenic mouse model. *Circulation,* 29.

Iwama, G.K., Afonso, L.O.B., Todgham, A., Ackerman, P., and Nakano, K, 2004. Are HSP suitable for indicating stressed states in fish? *J. Exp. Biol.*, 207: 15-19.

Keller, J.M., Escara-Wilke, F., and Keller, E.T, 2008. Heat stress-induced heat shock protein 70 expression is dependent on ERK activation in zebrafish (*Danio rerio*) cells. *Com. Biochem. and Physiol.,* Part A, 150: 307-314.

Landry, J., Chretain, P., Lambert, H., Hickey, E., and Weber, L.A, 1989. Heat shock resistance conferred by expression of the human HSP27 gene in rodent cells. *J. Cell. Biol.* 109: 7-15.

Mao, L., Bryantsev, A.L., Chechenova, M.B and Shelden, E.A, 2005. Cloning, characterization, and heat stress-induced redistribution of a protein homologous to human HSP27 in the zebrafish *Danio rerio*. *Exp. Cell Res.,* 306: 230-241.

Mehlen, P., Briolay, J., Smith. L., Diaz-Latoud, C., Fabre, N., Pauti, D., and Arrigo, A.P, 1993. Analysis of the resistance to heat and hydrogen peroxide stresses in COS cells transiently expressing wild type or deletion mutants of the Drosophila 27 kDa heat shock protein. *Eur. J. Biochem.,* 215: 277-281.

Morimoto, R.I., Tissieres, A., and Geogopoulos, C, 1990. Stress proteins in biology and medicine (New York: Cold Spring: Harbor Laboratory Press).

Multhoff, G, 2007. Heat shock protein 70 (HSP70): Membrane location, export and immunological relevance. *Methods,* 43: 229-237.

Osman, A.G.M., Al-Awadhi, R.M., Harabawy, A.S.A., and Mahmoud, U.M, 2010. Evaluation of the use of protein electrophoresis of the African Catfish *Clarias gariepinnus* (Burchell, 1822) for biomonitoring aquatic pollution. *Env. Res. Jour.,* 4(3): 235-243.

Sanders, B.M., Nguyren, J., Martin, L.S., Howe S.R., Coventry, S., 1995. Induction and subcellular localization of 2 major stress protein in response to copper in the head minnow *Pimephales promelas*. *Com. Biochem. and physiol.,* 112C: 335-343.

Sujatha, S., 1997. Effect of Temperature, Salinity and Starvation on the protein profile in the selected tissues of catfish *Mystus vittatus* (M.Sc., Dissertation submitted to Holy Cross College, Thrichirappalli-2.

Theodarakis, C.W., Surney, S.J.D., Bickharn, J.W., Lyne, T.B., Bardley, B.P., Hawkins, W.E., Farkas, W.L., Mc Carthy, J.F., and Shugart, L.R, 1992. Sequential expression of biomarkers in bluegill sunfish exposed to contaminant sediment. *Ecotoxicol.,* 1: 45-73.

Wang, J., Wei, Y., Li, X., Cao, H., Xu, M., and Dai, J, 2007. The identification of heat shock protein genes in goldfish (*Carassius auratus*) and their expression in a complex environment in Gaobeidian Lake, Beijing, China. *Com. Biochem. and Physiol.,* 145: 350-362.

****