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Effect of Organophosphate Pesticide, Chlorpyriphos on Biochemical Constituents In Snakehead Fish, Channa Gachua (F. Hamilton)

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ABSTRACT

Farmers still use the pesticides as the most effective weapon to protect the crop. Chlorpyriphos is most widely used organophosphate pesticide and being broad spectrum of harmful effects. Therefore present investigation aimed to study the effect of chlorpyriphos on biochemical constituents of fish, Channa gachua. In acute toxicity (96 hrs.) experiment the fish C. gachua exposed to LC0 and LC50 concentrations of pesticide. Significant depletion in glycogen content was observed in muscle, gill, liver, kidney and brain when compared to the control. The total protein content was decreased in all above tissues whereas fluctuations was also observed in lipid content. From the results it was concluded that the harmful pesticide caused significant depletion in biochemical composition of fish, this may be due to the utilization of these constituents for high energy demand, to cope up with pesticidal stress. Hence the harmful pesticide intoxication has made defective consequences in the normal metabolic pathways which led increasing the rate of mortality in fish population.

Keywords: Chlorpyriphos, Biochemical Constituents, Pesticides, Acute Toxicity

I. INTRODUCTION

People attempted to increase the world's food production to solve the problem of malnutrition, and overcome the basic need of population, this is achieved by the use of fertilizers to nourish the plants and use of pesticides to protect them from pests (Ganeshwade, 2011). Now a days large quantity of pesticides are used to control insect pest but these harmful chemicals entered into water bodies and pose a great threat to aquatic organisms. Chlorpyriphos is an organophosphate pesticide widely used in agricultural and domestic field to control of insect pests and vectors of different diseases. It acts on the nervous system of insects by inhibiting acetylcholinesterase. Its main break down products in the environment is chlorpyriphos oxon which is more toxic. Fishes are good indicator of aquatic pollution. Nutritional value of fish depends on their biochemical composition which is affected by water pollution.

Study of biochemical parameters like glycogen, protein and lipid helps to access the effect of harmful toxicant on vital tissues of fishes. Alterations in biochemical contents in different tissues of fish due to different pesticides have been reported by many workers e.g. Naveed et al. (2006) in fish, Channa punctatus exposed to lihocin, Sing et al. (2010) in fish, C. punctatus exposed to phorate, Ganeshwade (2011) in fish, Puntius ticto exposed to dimethoate, Stalin and Das (2012) in Cirrhinus mrigala exposed to fenthion, Magar and Dube (2013) in C. punctatus exposed to malathion, Chamarthi et al. (2014) in C. carpio exposed to quinalphos, Justin and Josef (2015) in Oreochromis mossambicus exposed to acetamipride.

Though the numerous workers have been reported the alterations in biochemical components, the level of depletion in biochemical constituents and the nature of toxicity is different according to species of fishes and type of pesticide. Therefore, the present investigation was designed to understand the effect of acute exposure of chlorpyriphos on glycogen, protein and lipid content in fish, C. gachua.

II. METHODS AND MATERIAL

A. Material:

1) Experimental fish - Live specimen of C. gachua of size15 ± 1 cm and weight 50 ± 5gm were obtained from Krishna river around karad city with the help of fisher man. The collected fish were kept in 1% solution of KMnO4 for some time to protect from dermal infection. Finally they were kept in glass aquarium and fed on commercial fish food. They were acclimatized in laboratory for 10 days at room temperature.

2) Pesticide - Commercially available organophosphorus pesticide chlorpyriphos was used for present research work. The pesticide brought from Local agro chemist shop. **B. Methods:**

1) Experimental set up – Under the experiment healthy fishes were divided in to three groups each group contained ten fishes. Group 1st was considered as control and group 2nd and 3rd as experimental groups. Fishes in the experimental groups were exposed to 35 ppm (LC0) and 50 ppm (LC50) concentration chlorpyriphos for 96 hrs. At the end of each exposure period the fishes were sacrificed and desired organs were quickly excised to study biochemical parameter by employing following methods.

2) Biochemical methods – i) Anthrone method was used to determine total glycogen content in tissues, (Siefter et al., 1950). ii) Total protein content was determined with Folin Ciocalteau reagent according to Lowry Method (Lowry, et. al., 1951). iii) Lipid were extracted by Floch, et al.(1957), and estimated by sulphophosphovaniline method (Barnes and Blackstock, 1973).

The results obtained in the present study were expressed as mean ± SD (standard deviation). The statistical differences between various groups were analyzed by the One Way ANOVA and the significance was observed at the $p>0.05$, $p<0.05$, $P<0.01$ and $p<0.001$ level.

III. RESULTS

In the present study the amount of glycogen, protein and lipid in some important organs like muscle, gill, liver, kidney and brain was estimated in control fish and in fishes exposed to lethal concentration of chlorpyriphos for 96 hrs. (acute). The calculated values of total glycogen, protein and lipid along with percent change in the different organs in control fish and fishes exposed acutely to different concentration of pesticides with standard deviation are shown in table 1-3.

A. Glycogen

The estimated values of the glycogen in control fish were 1.8512 mg in liver, 1.4318 mg in kidney, 1.2622 mg in brain, 0.8514 mg in muscle and 0.7588 mg in gill per gram wet weight of each tissue. Changes in glycogen content in different organs of the test fish exposed acutely to 35 ppm concentration of chlorpyriphos are shown in Table 1. Highly significant decrease ($P<0.001$) was observed in the glycogen content in the kidney (22.77) and liver (22.44) of fishes exposed to this concentration of chlorpyriphos. Moderately significant ($P<0.01$) decrease was observed in gill (23.54) followed by brain (18.53) and in muscle (22.46) the decrease was less significant ($P<0.05$) in comparison with other tissue. Whereas highly significant ($P<0.001$) depletion in the glycogen content was noticed in all the organs

of test fish exposed to 50 ppm concentration. The percent depletion was higher (70.53) in liver which was followed by gill (64.11), muscle (60), brain (59.27) and kidney (49.02) (Table 1).

Table 1. Showing changes in the glycogen (mg/gm. wet weight tissue) in different organs of control fish and in fishes exposed to different concentrations of chlorpyriphos at 96 hrs. exposure period (acute).

Organ	Muscle	Gill	Liver	Kidney	Brain
Control	0.8514 ±0.09	0.7588± 0.08	1.8512± 0.08	1.4318 ±0.08	1.2622 ± 0.07
35 ppm (LC0)	0.6967 ±0.08 (-18.17) *	0.5802 ±0.07 (-23.54) **	1.4411 ±0.08 (22.14) ***	1.1057 ±0.05 (-22.77) ***	1.0283 ±0.12 (18.53) **
50 ppm (LC50)	0.3405 ±0.05 (-60) ***	0.2723 ±0.05 (-64.11) ***	0.5454 ±0.06 (-70.53) ***	0.7298 ±0.06 (-49.02) ***	0.5518 ±0.06 (-59.27) ***

Table 2. Showing changes in the protein (mg/gm. wet weight tissue) in different organs of control fish and in fishes exposed to different concentrations of chlorpyriphos at 96 hrs. exposure period (acute).

Organ	Muscle	Gill	Liver	Kidney	Brain
Control	19.824± 0.69	24.228± 0.65	51.940 ± 0.91	37.130 ± 1.26	12.908 ± 0.50
35 ppm (LC0)	17.626± 0.24 (-11.08) ***	19.194± 1.35 (-20.77) ***	44.916± 0.96 (-13.62) ***	31.656± 1.32 (-15.19) ***	10.096± 1.13 (-23.33) ***
50 ppm (LC50)	09.244± 0.73 (-53.36) ***	11.536± 0.69 (-52.38) ***	32.504± 0.81 (-36.54) ***	19.952± 0.84 (-46.26) ***	6.676± 0.72 (-48.28) ***

Table 3. Showing changes in the lipid (mg/gm. wet weight tissue) in different organs of control fish and in fishes exposed to different concentrations of chlorpyriphos at 96 hrs. exposure period (acute).

Organ	Muscle	Gill	Liver	Kidney	Brain
Control	3.908 ± 0.05	57.68 ± 0.12	3.499 ± 0.18	4.620 ± 0.09	5.895 ± 0.15
35 ppm (LC0)	3.292 ± 0.17 (-18.70) ***	5.349 ± 0.28 (-7.82) **	3.561 ± 0.12 (1.75) NS	4.228 ± 0.22 (-9.26) **	5.493 ± 0.18 (-7.30) **
50 ppm (LC50)	2.601 ± 0.63 (-33.43) ***	4.382 ± 0.10 (-24.02) ***	3.896 ± 0.10 (11.36) **	3.587 ± 0.16 (-22.35) ***	4.824 ± 0.16 (-18.16) ***

Values are mean ± SD, n=5, figures in parenthesis are percentage Decrease over control.

Values significant at NS = $P > 0.05$ (Non-significant), * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

Protein

The estimated values of the protein in control fish were 51.940 mg in liver 37.130 mg in kidney, 24.228 mg in gill, 19.824 mg in muscle and 12.908 mg in brain per gram wet weight of each tissue. Highly significant depletion in protein ($P<0.001$) in all tissues was observed in fishes exposed to 35 ppm concentration of this pesticide. In brain (24.18) percent depletion more pronounced which was followed by gill (20.77) and kidney (15.19). The percent depletion of protein in liver (13.62) and muscle (11.28) was less than other tissues. But, the fishes exposed to 50 ppm concentration of chlorpyriphos for 96 hrs. showed drastic depletion in protein. The decrease in protein was also highly significant ($P< 0.001$) in all tissues in muscle the percent depletion (53.36) was higher, followed by gill (52.18), brain (48.28), and kidney (46.26). In liver (36.54) depletion in protein content was less than other tissues (Table 2).

Lipid

The estimated values of the lipid was 5.895 mg in brain, 5.768 mg in gill, 5.260 mg in intestine, 4.658 mg in stomach, 4.620 mg in kidney, 3.908 mg in muscle and 3.499 mg in

liver per gram wet weight of each tissue. The lipid content in all tissues was decreased after exposure of fishes to 35 ppm concentration of this pesticide except liver. In muscle lipid content was decreased more significantly ($P<0.001$) and the decrease was 18.70. The percent depletion in kidney, gill and brain was moderately significant which was 9.26, 7.82 and 7.30 respectively. But in liver non-significant increase in lipid content (1.75) was observed at this concentration. In fishes exposed to 50 ppm (LC 50) concentration of this pesticide highly significant ($P<0.001$) decrease in lipid content was observed in all tissues except liver. The percent depletion in muscle was higher (33.43) than other tissues. In gill the percent depletion was 24.02 followed by kidney (22.35) and brain (18.16). However, moderately significant ($P< 0.01$) increase was observed in liver (11.36) as compared to control (Table 3).

In all the organs the glycogen content was found decreased significantly as compared to the control fish, but percent depletion was more in fishes exposed to LC 50 concentration than the LC0.

III. DISCUSSION

The contamination of water by widely utilized organophosphate pesticide such as chlorpyriphos is a potential problem for fishes and aquatic organism. In present study attempt has been made to study acute effect of chlorpyriphos on biochemical composition of tissues like muscle, gill, liver, kidney and brain of fresh water fish C. gachua. The biochemical constituents are source of energy in animal body is present in the form of stored energy which is used in starved and stressed condition. Glycogen is the prime and important biochemical constituents in tissues of animal. It acts as building blocks and reservoir of chemical energy in the cell which can increased or decreased according to organismal need (Kumar and Ali, 2013). Glycogen provides the energy for the animal which is essential for performing different process (Lehninger, 2004). Carbohydrates are mainly stored in liver of animal in the form of glycogen and it is exported in the form of hexose units for maintenance of blood glucose and readily available for glycolysis (Herper, 2003). Protein serves as an alternative source of energy for animal when the insufficient energy is available from the other sources like carbohydrates and lipids. Protein is the building blocks of animal body. It is the most important constituents for growth, development and maintenance of life (Waghmare and Wani, 2014). Protein plays an important role in cellular metabolism and regulates intra and extra cellular interactions media as a part of cell membrane and enzyme (Anita and Venkata Rathnamma, 2016). All cells contain lipid in the form of globules scattered in the cytoplasm (Tamizhazhagan et al., 2016). Lipid is an essential biochemical constituent in tissues of all animals, and plays vital role in the energy metabolism. (Kumar and Ali, 2013). Lipids are also the strong form of energy like glycogen. Lipid form an essential component of protoplasm and during extreme starvation considerable amount can be extracted from tissues.

Due to pesticidal stress, such prime and important energy source is affected significantly and alters various processes in the fish. The biochemical constituents like glycogen, proteins and lipids are important to indicate the susceptibility of organs system to pollutants (Verma and Tonk, 1983). The results obtained in the present work showed fluctuations in the glycogen, protein and lipid content in muscle, gill, liver, kidney, and brain of fish, C. gachua exposed to lethal concentrations of

chlorpyriphos at acute exposure period. The alterations were time of exposure and concentration of pesticides dependent. Similar results have also been observed by Venkataramana et al. (2006) in cardiac muscle of Glossogobius giuris exposed to malathion, Thenmozhi et al. (2011) in liver , muscle and gill of L. rohita exposed to malathion, Veeraiah et al. (2013) in gill, muscle, brain, liver and kidney of fish, Labeo rohita after sublethal exposure of indoxacarb, by Tripathi and Yadav (2015) in gill, muscle, brain, liver, kidney and gut of L. rohita exposed to phenthoate, by Pechiammal and Kiruthika (2016) in gill liver kidney and muscle of Cirrhinus mrigala exposed to rogor, by Tamizhazhagan et al. (2016) in muscle, liver and kidney of L. rohita exposed to monochrotophos, by Verma and Rawat (2017) in ovary of Heteropneustes fossilis exposed to chlorpyriphos, by Padmavathi et al. (2017) in muscle and liver of Mystus vittatus exposed to cypermethrin. The biochemical changes occurring in the body of the organisms give first indication of stress. Here, in present investigation depletion in glycogen content was might be due to the rapid utilization of stored glycogen for energy production in pesticide stressed condition at acute exposure. Required Energy in the form of ATP might be produced due to anaerobic breakdown of glycogen which may leads to breakdown of more amount of glycogen to cope up the energy need under stress condition. A fall in glycogen levels indicates its rapid utilization to meet the enhanced energy demands in pesticide treated animals through glycolysis or hexose monophosphate pathway (Cappon and Nicholas, 1975). Pesticides are known to act on endocrine system (Edwards, 1973). Hence, it contributes to the decreased glycogen synthesis. Decreased glycogen synthesis is also attributed to the inhibition of the enzyme glycogen synthatase which mediates glycogen synthesis.

The decreasing trend in the protein content as observed in the present study in all tissues may be due to metabolic utilization of the ketoacids to gluconeogenesis pathway for the synthesis of glucose; or due to the directing of free amino acids for the synthesis of necessary proteins, or for the maintenance of osmotic and ionic regulation (Schmidt Nielson, 1975). According to Kamble (1999) these toxic compounds inhibit the incorporation of amino acids into proteins and increased degradation of protein into amino acids. This phenomenon supports by Pugazhedy et al. (2012), according to them reduction in protein might be due to the blocking of protein synthesis, protein denaturation or interruption in the amino acid synthesis under pesticidal stress. Protein might be used in cell repair, tissue organization and formation of lipoproteins, which are important cellular constituents of cell membranes and cell organelles present in the cytoplasm.

The depletion in lipid level might be due to the lipolysis in different tissues to overcome the high energy demand by the fish under pesticidal stress, rate of lipolysis might be accelerated for the production of energy and subsequently used for glucose synthesis. On the other hand it is suggested that the lipid synthesis may be inhibited and mobilization of stored lipids through β-oxidation and unsaturation of lipid molecules (Jha, 1991). Pesticide may affects on cellular structure of the tissue and lipid might be used in cell repair and tissue organization due to that depletion in lipid content may occur. The increased lipid content in liver may be due to cellular degeneration in liver results into collapsed metabolic activity which inhibit emulsification of lipids. As the liver is commonest site for accumulation of lipids it plays central role in lipid metabolism. The accumulation of lipid might be due to mobilization of lipids to the liver for metabolism and energy production but in liver the excess lipids are saturated (i.e. hyperlipidemia) exceeding capacity of the liver to metabolize it (Harsh Mohan, 2010). Pesticides and their metabolites interferes with the enzymes involved in the metabolic pathway, they exerts their effect on synthesis of biochemical

compound and stored biochemical compounds in the form of energy utilized to cope with pesticidal stress on metabolism and physiology of fish.

IV. CONCLUSION

Present study revealed that the pesticide chlorpyriphos is potent to cause toxic responses, and biochemical alterations in fish. The natural physiological functioning of an organisms gets distributed on exposure to toxicants, it induces its effect first at cellular or even at molecular level, but ultimately leads into physiological, pathological and biochemical alteration. Pesticides even in minute extent, causing a stress to aquatic organisms which reflected by the behavioral, biochemical and pathological changes and at the end death of the animal was evident. But an agricultural efforts reducing the use of pesticides and implementing natural remedies for pest control can become one solution for pesticidal pollution.

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