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Sublethal Toxicity of Cyperkill (A Synthetic Pyrethroid Pesticide) on Glycogen Content in the Tissues of *Labeo rohita*

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KEYWORDS

Labeo rohita, Glycogen, Cyperkill, Synthetic pyrethroid, Toxicity.

A B S T R A C T

The aim of the present investigation was to study the toxic effect of a synthetic pyrethroid cypermethrin on glycogen levels in vital tissues (gill, muscle, brain, liver and kidney) *Labeo rohita* after exposure to sub lethal concentrations (0.226, 0.453, 0.679 and 0.906µg/L representing 5, 10, 15 and 20% of 96h LC_{50} value i.e 4.53µg/L) of cypermethrin for three different exposure periods, 1, 2 and 3 weeks. It was found that the glycogen content was reduced in all the tissues under sub-lethal concentrations of cypermethrin at 1, 2 and 3 weeks. Reduction was observed in all the tissues tested at all the concentrations and exposure periods. Reduction in glycogen content was maximum (45%) in liver and minimum (13.08%) in brain at 0.906µg/L at 21 days. It was observed that all the alterations were dependent on concentration as well as exposure period.

Introduction

Injudicious and indiscriminate use of pesticides in agri and aqua practices results in the contamination of surface and ground waters by drift, runoff, drainage and leaching (Cerejeira *et al*., 2003) and contamination of aquatic ecosystems such as lakes, reservoirs, wetlands, rivers, ponds, paddy-fields, streams, and other low-lying areas has erupted as global problem in recent years and is a topic of great concern worldwide (Prusty *et al*., 2015; Toumi *et al*., 2013). Pesticides of various categories viz., organophosphates, organochlorines, pyrethroids and carbamates are used to preserve the standing crops from the attack of pests and to increase the crop production, in order to meet the ever-increasing food demand (Chandola *et al*., 2011; Gupta *et al*., 2012). Pyrethroids have emerged as good substitutes for organochlorine, organophosphate and carbamate insecticides due to their high bio-efficacy, biodegradable and lower toxicity to mammals and birds (Parvez and Raisuddin, 2006). They have been found to be neurotoxic and lethal to fish at concentrations up to 1000 times

lower than the corresponding values for animals and birds (Koprucu and Aydin, 2004).

The growing use of synthetic insecticides is intensifying global pollution risks. Insecticides are toxic and were designed to repel or kill unwanted organisms and when used for their different purposes they may be brought to water bodies killing or influencing the lives of aquatic organisms (Sayed *et al*., 2007). Biochemical parameters such as glucose, protein, and glycogen etc. are considered as essential indicative markers that give vital information about the internal environment of the animals (Authman *et al*., 2013; Saravanan and Ramesh, 2013) and frequently used when fish physiology is connected to analyze and comprehend the toxicological impact of external stressors and harmful chemicals (Osman *et al*., 2010). Fish are one of the most important aquatic species because of their economic value and their sensitivity to contaminants, and have been used in a wide range of biological assays (Huang *et al*., 2014). It can last for more than one month without feeding and are more suitable for long-term monitoring of accidental discharges of various toxic materials into aquatic environments (Ren and Wang, 2010). A review of literature indicates that biochemical changes in fish under cypermethrin exposure were extensively reported (Neelima *et al*., 2015a, b & c; Elaimy *et al*., 2014; Olalekan 2014; Neelima *et al*., 2013; Gabriel *et al*., 2012; Firat *et al*., 2011; Neelima *et al*., 2011a & b; Singh *et al*., 2010). However, it is relevant that data on sulethal toxic effects of cypermethrin using *Labeo rohita* as experimental model has been scarcely studied. Hence, in this present investigation, an attempt has been made to study the alternations in glycogen content in *Labeo rohita* under the toxic effect of a synthetic pyrethroid, cypermethrin.

Materials and Methods

Labeo rohita with length 7.5 to 8.5 cm, weight 6.5 to 8.0g, irrespective of their sex were obtained from Ratna Singh Hatcheries, Nandivelugu, Guntur (A.P), India. The fish were acclimatized to the laboratory conditions in large plastic water tanks for 15 days at 28±1°C. Water was renewed daily with12-12 h dark and light cycle. During the period of acclimatization, the fish were fed with groundnut oil cake and rice bran. Feeding was stopped one day prior to the biochemical analysis. All the precautions laid by committee on toxicity tests to aquatic animals (APHA, 1998) were followed and such acclimatized fish only were used for the study. If mortality exceeded 5% in any batch of fish during acclimatization, the entire batch of that fish were discarded. Fish were exposed to 4 sub-lethal concentrations of cypermethrin (Trade name: Cyperkill) (0.226, 0.453, 0.679 and 0.906µg/L representing 5, 10, 15 and 20% of 96h LC_{50} value i.e 4.53µg/L) for a period of 1, 2 and 3 weeks. At the end of the exposure period, fish were sacrificed to death and target organs such as gill, muscle, brain, liver and kidney were dissected out and **t**he glycogen was estimated by the method of Kemp *et al.*, (1954) and is expressed as mg of glycogen / g wet weight of the tissue.

Results and Discussion

Calculated values for glycogen content along with standard deviation are given in table 1. Percent change of glycogen in experimental fish over control is graphically represented in figure 1. In the tissues of control fish, total glycogen content was in the order of liver > gill > kidney > muscle > brain. In experimental fish, there was a gradual decrease in glycogen content in all the tissues under sub-lethal concentrations of cypermethrin at 1, 2 and 3 weeks. In the

control fish, the liver glycogen levels were in the range of 83.10±1.16 – 83.27±1.28 mg/g wet wt. When the fish exposed to 0.906µg/L of cypermethrin, there was a decrease in the liver glycogen levels to 62±0.98, 52.94±0.48 and 45±1.98 mg/g wet wt after 7, 14 and 21 days of exposure periods respectively. Maximum decrease of 45.84% over control was observed in liver at 0.906µg/L after 21 days of exposure period. In similar manner, gill, kidney, muscle and brain glycogen levels were also found to decrease as the concentration of the pesticide and exposure period increases. Brain showed glycogen levels of 17.33±2.42 mg/g wet wt. in control fish. These levels were found to decrease to 13.08±2.57 mg/g wet wt. equals to 24.52% enhancement over control. Among the tissues studied maximum decrease in glycogen levels was observed in liver (45.84%) followed by gill (39.48%), kidney (37.51%), muscle (35.12%) and brain (24.52%).

Glycogen is a sub divisional polysaccharide and the major storage of glucose that serve as a form of energy in animals. It plays an important role in the glucose cycle that can be quickly mobilized to meet a sudden requirement for glucose. The glycogen content in various tissues of *Labeo rohita* was decreased with increased toxicant concentration in the present experiment. Glycogen levels are found to be the highest in liver tissue of all fish, as it is chief organ of carbohydrate metabolism in animals. Liver glycogen is concerned with storage and export of hexose units for maintenance of blood glucose and that of muscle glycogen is to act as a readily available source of hexose units for glycolysis within the muscle itself. Glycogen depletion is more prevalent under hypoxic conditions that may stimulate phosphorylase activity bringing about a drop in glycogen level. In general, when animal undergone to stress and strain, then the glycogen reserves were used as substitution of metabolic requirement to meet the energy demands through glycolysis or hexose monophosphate shunt pathway. Depletion of glycogen content in all the tissues might be due to the utilization of carbohydrates for energy production as a result of cypermethrin induced hypoxia. In the present study, glycogen, which is ultimate energy source, decreased resulting in higher demand for carbohydrate and their precursors to keep the glycolytic and TCA cycle at sustained levels to cope the energy demands for both physical activities and enhanced physiological processes of metabolizing and eliminating the toxicant during pesticide stress condition in test animal. It is assumed that decrease in glycogen content may be due to the inhibition of hormones which contribute to glycogen synthesis. According to Datta and Kaviraj, 2003, stress caused by cypermethrin resulted in an increase in the synthesis of adrenocorticotropic hormone and glucagon and decrease in the synthesis of insulin. Thereby, hepatic glycogen is rapidly converted into glucose and passes into systemic circulation ever-increasing the blood glucose level. Though brain tissue is metabolically active, lower glycogen content was observed, since it lacks the inherent potential to store glycogen and is dependent on blood glucose for all its metabolic activities (Lehninger, 2008).

Glycogen depletion in the tissues studied after cypermethrin stress has been reported in several studies with fish (Olalekan, 2014; Veeraiah *et al*., 2003; Patil and Patole, 2012; Korkmaz *et al*., 2009; Luther Das *et al*., 1999; Tiwari *et al*., 2012; Saha and Kaviraj, 2009; Begum 2005; Reddy and Yellamma, 1991a & b). The significant decrease in liver, the vital organ and the site of the metabolism induces the toxicant effect

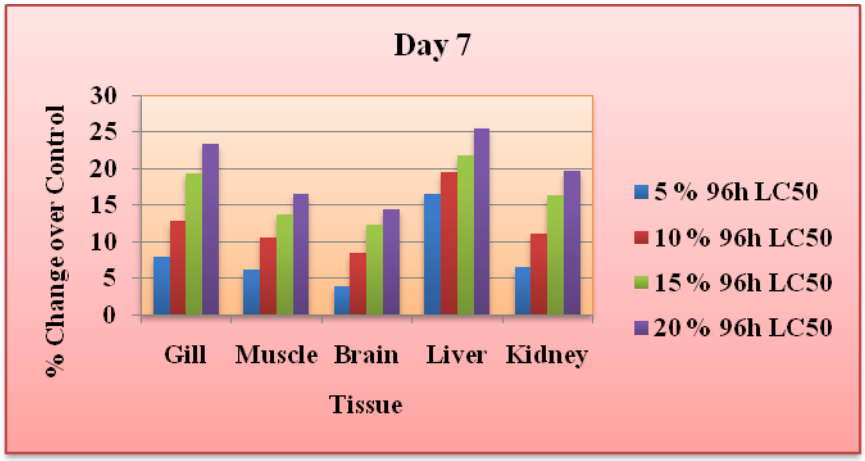
overall affecting the life processes, especially growth and reproduction. In other organs, it will lead to the disturbance in organ coordination and ultimately and definitely cannot lead a normal life.

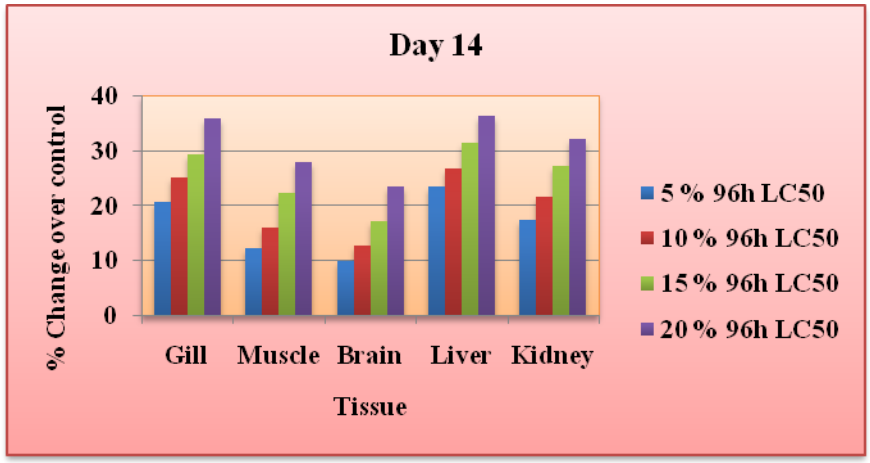
Table.1 Changes in the Glycogen content (mg/g wet weight of the tissue) in different tissues of *Labeo rohita* exposed to sublethal concentrations of cypermethrin (25% EC)

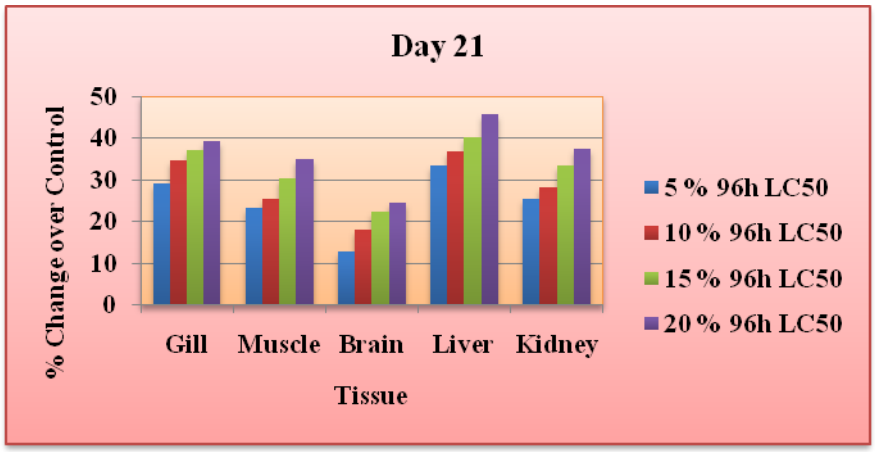
Exposure period in Weeks	Tissue	Concentration of Cypermethrin (µg/L)				
		Control	0.226	0.453	0.679	0.906
1	Gill	52.16 ± 1.30	48.04 ± 0.24	45.45 ± 2.59	42.09 ± 1.41	40.00 ± 0.29
	Muscle	25.08 ± 1.40	23.53 ± 2.84	22.44 ± 1.54	21.65 ± 0.96	20.94 ± 1.28
	Brain	17.38 ± 0.62	16.70 ± 1.42	15.92 ± 0.18	15.25 ± 2.57	14.86 ± 1.54
	Liver	83.27 ± 1.08	69.52 ± 1.72	67.07 ± 2.58	65.13 ± 1.84	62.00 ± 0.98
	Kidney	44.38 ± 0.62	41.50 ± 1.20	39.46 ± 0.29	37.08 ± 2.98	35.63 ± 1.34
2	Gill	52.10 ± 2.30	41.28 ± 2.64	38.96 ± 1.90	36.82 ± 0.48	33.37 ± 1.58
	Muscle	25.05 ± 1.42	21.98 ± 2.48	21.05 ± 0.35	19.43 ± 2.48	18.05 ± 0.35
	Brain	17.35 ± 0.72	15.60 ± 0.18	15.12 ± 0.18	14.38 ± 2.28	13.26 ± 0.57
	Liver	83.14 ± 2.06	63.51 ± 2.85	60.81 ± 1.56	57.04 ± 0.48	52.94 ± 0.48
	Kidney	44.26 ± 0.44	36.58 ± 0.19	34.67 ± 0.29	32.22 ± 2.39	30.00 ± 1.57
3	Gill	52.12 ± 2.30	36.84 ± 1.28	34.08 ± 0.48	32.75 ± 2.34	31.54 ± 1.61
	Muscle	25.05 ± 0.82	19.22 ± 2.10	18.65 ± 2.48	17.41 ± 0.60	16.25 ± 1.21
	Brain	17.33 ± 2.42	15.08 ± 0.18	14.21 ± 2.34	13.45 ± 1.19	13.08 ± 2.57
	Liver	83.10 ± 1.16	55.36 ± 1.75	52.44 ± 0.48	49.73 ± 2.66	45.00 ± 1.98
	Kidney	44.22 ± 1.24	33.00 ± 1.57	31.70 ± 1.72	29.41 ± 2.33	27.63 ± 0.49

Mean± standard deviation, n=5, Values are significant at $p < 0.05$

Fig.1 Per cent change over control in the Glycogen content (mg/g wet weight of the tissue) in different tissues of *Labeo rohita* exposed to sublethal concentrations of cypermethrin (25% EC)







Olalekan (2014) reported that *Clarias gariepinus* exposed to cypermethrin (20µg/L) for 5 days showed significant decrease in glycogen levels of tissues of the liver and muscle. Veeraiah *et al*., 2013 reported *Cirrhinus mrigala* exposed to lethal and sub-lethal concentration [96h LC_{50} (2.28ppm) and $1/10^{th}$ of 96h LC_{50}] of cypermethrin (10% EC) for 96h showed decrease in total glycogen over control. Significant decrease in glycogen was reported by Patil and Patole (2012) in *Lepidocephalichthys guntea* exposed to sub-lethal concentrations ($1/4^{th}$ and $3/4^{th}$ of LC_{50}) of cypermethrin for 96h. Tiwari *et al*. (2012) revealed sub-lethal doses of cypermethrin (0.129µg/L, 0.258µg/L for 24h and 0.082µg/L, 0.164µg/L for 96h exposure period caused significant reduction in liver and muscle glycogen in *Labeo rohita*.

Saha and Kaviraj (2009) observed decline in the level of glycogen in *Heteropneustes fossilis* due to cypermethrin (0.3 - 0.5µg/L). Depletion of hepatic glycogen due to cypermethrin treatment was also observed in the fish, *Clarias batrachus*. Luther Das *et al.* (1999) studied the effect of cypermethrin 25% EC on biochemical composition and observed marked decrease in glycogen content in the gills of *Channa punctatus*. Reddy *et al.*, (1991a) reported significant decrease in glycogen content in liver, brain and gill tissues of *Tilapia mossambica* exposed to sub-lethal concentration (0.04 ppm) of cypermethrin. Reddy *et al.* (1991b) reported significant changes in carbohydrate metabolism in liver, brain and gill tissues of *Tilapia mossambica* exposed to sub-lethal concentration of 0.04 ppm cypermethrin. They calculated 24h LC_{50} as 0.2 ppm and

observed a decrease in glycogen levels in all tissues.

Conclusion

From the present study, it is concluded that the decrease in glycogen content in the vital tissues of the test animal emphasized the signs of cypermethrin toxicity, and it is in agreement with the previous findings in which glycogens is inhibited with the increasing concentrations of the test chemicals. As a result of its toxic effect on glycogen levels, it is concluded that test fish *Labeo rohita* is more sensitive to synthetic pyrethroid pesticides such as cypermethrin.

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