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Changes in the Protein Content (Mg/Gr Wet Weight of the Tissue) in Different Tissues of Channa Punctatus on Exposure to Sub-Lethal and Lethal Concentrations of Chlorpyrifos Technical Grade For 24H

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Abstract
The protein content was significantly decreased in liver, kidney and muscle stated that the depletion of tissue protein content after 7 days exposure period may be due to increased proteolysis. This in turn will contribute to the presence of excess free amino acids which are directed to the tricarboxylic acid (TCA) cycle and further effective utilization of its products for metabolic activity. Under exposure to sub-lethal and lethal doses of chlorpyrifos technical grade and 20% EC, the total protein content was found to be decreased in all the tissues tested. Maximum decrease was noticed in gill and liver followed by muscle, brain and kidney at 8 days sub lethal exposure. In lethal 96 h exposure significant decrease was noticed in all the tissues. Maximum decrease was noticed in gill and liver followed by muscle, kidney and brain. The apparent decrease of total protein content under sub lethal and lethal exposure of chlorpyrifos may be due to the detoxification of enzymes. The decreased tendency of total proteins may also be due to metabolic utilization of the ketoacids in gluconeogenesis pathway for the synthesis of glucose or may be due to directing the synthesis of proteins from free amino acids (Schmidt Nielson, 1975). The decrease in protein content was more due to breakdown rather than retarded synthesis which is supported by the findings of Radhaiah (1988).

Keywords: Channa punctatus, chlorpyrifos, oxygen consumption.

Introduction

Objective: To know the Changes in the Protein content in different tissues of Channa punctatus on exposure to sub-lethal and lethal concentrations of chlorpyrifos technical grade for 24h

Methodology

For the estimation of total protein content of liver, muscle, brain, gill, and kidney of the exposed test fish, the modified method of Lowry *et al*., (1951) was followed:

Five percent of the homogenized tissue of gill, muscle and brain and two per cent homogenized tissue of of liver and kidney were made with five per cent trichloroacetic acid and were centrifuged for 10 min at 3000 rpm. The supernatant was removed. The protein residue was suspended in the 1 ml of 1N NaOH, from which 0.2 ml of the extracted sample was taken into the test tube and was added with five ml of alkaline copper solution (50 ml of 2% $NaCO_3$ and 1ml of 0.5% $CuSO_4$. $5H_2O$ along with 1% sodium potassium tartrate) was mixed. The contents of the mixture stirred well and kept aside for a while for 10 minutes. For this, about 0.5 ml of folin phenol reagent (diluted with distilled water 1:1 ratio) was mixed. Waiting for a period of 30 minutes to develop colour, the optical density in a spectrophotometer against a blank was measured at 540 nm. The standard bovine serum albumin supplied by Sigma chemical Company, U.S.A was used to plot standard graph by the method of Lowry *et al*., (1951). The values obtained were reported as mg/g wet weight of the tissue.

Result

The values that were calculated for total proteins and per cent change over the control fish along with standard deviation were presented in Table 1.

In the control fish Channa punctatus, the total protein content was in the order of:

Technical Grade

24 h: Kidney >Liver > Brain > Gill > Muscle

The changes in protein distribution show gradual difference in metabolic calibers of various tissues. Because of its metabolic potential, the liver is being oriented towards synthesis of proteins and is the seat for the synthesis of various proteins, and it will also act as the regulating centre of metabolism and hence it is also much in proteins.

Under exposure to sub-lethal and lethal concentration of chlorpyrifos technical grade, for 24h, the total protein content was found to decrease in most of the tissues and the leotropic series in terms of decrement in protein content is:

24 h Technical Grade

Lethal: Liver > Muscle > Kidney > Gill > Brain

Sub-lethal: Liver > Kidney > Muscle > Gill > Brain

The decrease in glycogen content of the muscle of the test fish under exposure to lethal and sub-lethal doses of chlorpyrifos technical was evident. In all the tissues of the test organs i.e. muscle, brain, gill and kidney after exposure at lethal and sub-lethal doses of both technical grade chlorpyrifos, a decrement in glycogen content was noticed at the exposure periods.

The earlier reports on the impact of insecticides on carbohydrate metabolism in several fish species shows decline of the energy reserve under insecticide stress (Holden, 1974; Kabeer *et al*., 1979; Radhaiah, 1988; Rama Murthy, 1988). This indicates that exposure to chlorpyrifos causes in enhancement of energy demand. The glycogen content might be utilized more to overcome the extra requirement of energy due to stress conditions, which leads to the decrement in glycogen level. Endosulfan 96 hrs exposure decreased the glycogen level in crab Barytelphusa guerni (Nagender Reddy *et al*., 1991), in Clarias batrachus (Asfia Parveen *et al*., 1990) and also in Anabas scandens (Yasmeen *et al*., 1991).

The decrement on the content of glycogen in the liver and muscle tissue of Atlantic salmon was reported under fenvalerate sub-lethal exposure by (Haya, 1989), Hexachloro cyclo hexane (HCH) on freshwater fish Channa punctatusby (Ganathy *et al*., 1994); under exposure to monocrotophos on freshwater crab Barytelphusa guerni (Venkateswarlu and Sunita, 1995); under the doses of Heptachlor on Swiss albino mice (Nagabhushanam *et al*., 1994) and under phosphamidon exposure to Gambusia affinis (Govindan *et al*., 1994). Under sub-lethal doses exposure of cypermethrin causing decrease of glycogen in Tilapia mossambica (Reddy and Yellamma, 1991).

Table 1: Changes in the Protein content (mg/gr wet weight of the tissue) in different tissues of Channa punctatus on exposure to sub-lethal and lethal concentrations of chlorpyrifos technical grade for 24h.

S. No.	Tissue	Control	Sub-lethal	Percent change	Lethal	Percent change
1	Gill	126.48±3.14	102.18±0.98	19.21	89.36±2.73	29.34
2	Brain	134.82±3.02	112.64±2.17	16.45	98.54±4.50	26.90
3	Liver	190.26±3.17	138.62±1.26	27.14	108.92±3.79	42.75
4	Kidney	84.38±2.05	65.14±0.83	22.84	54.26±1.38	35.69
5	Muscle	186.2±5.64	144.82±2.73	22.25	112.84±1.41	39.39

The values of the results are the mean values of five observations

Standard Deviation is shown as (±)

The values are significant at $p < 0.05$

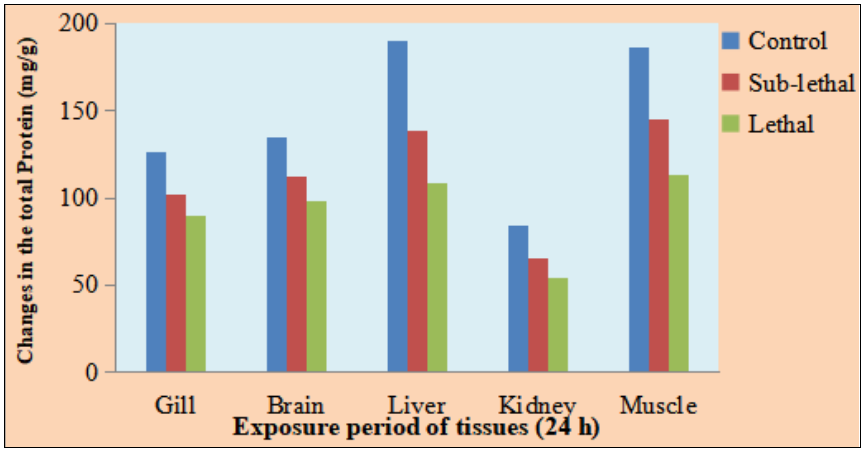

Fig 1: Changes in the Protein content (mg/gr wet weight of the tissue) in different tissues of Channa punctatus on exposure to sub-lethal and lethal concentrations of chlorpyrifos technical grade for 24h.

Labeo rohita reported by (Sridevi, 1991; Anuradha, 1993; Veeraiah and Durga Prasad, 1998; Veeraiah, 2001) in Cyprinus carpio (Ravisankar *et al*., 1992) and in Channa punctatus (Luther Das *et al*., 1999). Helinmayer *et al*., (1970) and Smart (1978) reported that the alteration in the enzymes associated with carbohydrate metabolism may result in toxic stress condition. This has caused decrease in glycogen level and is also attributed to the conversion of carbohydrates into aminoacids (Gaiton *et al*., 1965). Koundinya (1979) stated that the decrease in glycogen is due to stepped up glycogenolysis. Under exposure to malathion, similar changes were noticed in Heteroneustis fossilis (Kabeer *et al*., 1983), sumithion (Koundinya and Rama Murthy, 1978), endosulfan (Vasanthi and Ramaswamy, 1987) and in Channa striatus exposure to metasystox exposure (Natarajan, 1981).

In the present study also, it was noticed that under exposure to lethal and sub-lethal doses of chlorpyrifos technical grade caused decrease in the total glycogen level of the fish Channa punctatus due to toxic stress, causing the disruption of enzymes associated with metabolism of carbohydrates.

Conclusion

This study is useful to know the effect of pesticides from crops to biota and to human being through bio magnification.

In conclusion, the study on the changes in protein content (mg/g wet weight of tissue) in various tissues of Channa punctatus exposed to sub-lethal and lethal concentrations of chlorpyrifos technical grade for 24 hours highlights the significant impact of this pesticide on the physiological state of the fish. Exposure to chlorpyrifos resulted in a marked alteration in the protein content across different tissues, indicating the pesticide's potential to disrupt metabolic processes. The observed reduction in protein content in tissues exposed to lethal concentrations suggests severe physiological stress, likely due to the inhibition of protein synthesis and increased proteolysis. Conversely, sub-lethal concentrations induced less severe changes, which could still have long-term ecological implications. These findings emphasize the toxicological risks posed by chlorpyrifos to aquatic organisms and underscore the importance of regulating pesticide concentrations to minimize environmental harm. Further studies on the molecular mechanisms behind these changes could provide valuable insights into the adaptive responses of Channa punctatus to pesticide exposure. In conclusion, the study examining the changes in protein content (mg/g wet weight of tissue) in different tissues of Channa punctatus after exposure to sub-lethal and lethal concentrations of chlorpyrifos technical grade for 24 hours reveals significant disruptions in the biochemical and physiological processes of the fish. The data show that exposure to both concentrations of chlorpyrifos led to a reduction in protein content in key tissues, with a more pronounced decrease observed in the lethal concentration group. This suggests that chlorpyrifos exerts a toxic effect by impairing protein synthesis, which is critical for cellular function and overall metabolism.In the tissues exposed to lethal concentrations of chlorpyrifos, the decrease in protein content could be attributed to a combination of direct cellular damage, oxidative stress, and activation of proteolytic pathways. These changes may reflect the organism's attempt to cope with the toxicant, leading to protein breakdown and the potential loss of essential cellular functions. The sub-lethal concentrations, while inducing less severe effects, still resulted in measurable decreases in protein content, indicating that even low-level exposure can lead to significant physiological stress and potential long-term consequences for the health of the fish. The observed tissue-specific variations in protein content may also suggest that different tissues have varying sensitivities to chlorpyrifos toxicity, with some tissues possibly being more prone to oxidative damage or metabolic disruptions than others. These results highlight the complexity of pesticide toxicity, where multiple factors including concentration, exposure duration, and tissue-specific responses come into play. This study underscores the importance of understanding the broader ecological impacts of pesticides like chlorpyrifos, which are commonly used in agriculture but can pose significant risks to aquatic ecosystems. The findings also emphasize the need for more stringent regulation and monitoring of pesticide concentrations in aquatic environments to protect fish and other aquatic organisms from the harmful effects of chemical pollutants. Furthermore, future research into the molecular mechanisms underlying these protein changes, such as the roles of specific enzymes and stress-related proteins, could provide deeper insights into how Channa punctatus and other aquatic species adapt to or suffer from pesticide exposure.

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