

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 48h

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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. The decrease in protein content was more due to breakdown rather than retarded synthesis which is supported by the findings of Radhaiah (1988). 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, and 20% EC for 24, 48 and 96 h, the total protein content was found to decrease in most of the tissues and the leotropic series in terms of decrement in protein content

Keywords: *Channa punctatus*, Chlorpyrifos, Protein

INTRODUCTION

Toxicology is a multidisciplinary science that deals with the study of adverse effects of chemical, biological, and physical agents on living

organisms. In recent decades, rapid industrialization, urbanization, and intensified agricultural practices have led to the extensive use of chemical pesticides. As a result, these toxic substances increasingly contaminate aquatic ecosystems, posing serious threats to non-target organisms. Among commonly used pesticides, organophosphates such as chlorpyrifos are widely applied due to their effectiveness; however, their persistence and toxicity raise significant environmental concerns.

Aquatic ecosystems serve as the final recipients of many pollutants, including pesticides, heavy metals, and industrial effluents. These contaminants enter water bodies through surface runoff, leaching, and improper disposal, leading to both acute and chronic exposure in aquatic organisms. Fish are particularly vulnerable to such toxicants and are considered reliable bioindicators of environmental pollution. The freshwater fish *Channa punctatus* is frequently used in toxicological studies because of its ecological importance, sensitivity to pollutants, and suitability for laboratory experimentation. Biochemical constituents such as proteins and glycogen are vital for maintaining normal physiological functions. Proteins play a central role in cellular structure, enzyme activity, transport mechanisms, and regulation of metabolic pathways. Glycogen acts as an immediate energy reserve and is mobilized during stress conditions to meet increased metabolic demands. Any disturbance in these biochemical parameters reflects alterations in metabolic processes and provides early indications of toxic stress.

Exposure to pesticides like chlorpyrifos induces a variety of biochemical, physiological, and behavioral changes in fish. Under toxic stress, organisms tend to utilize their stored energy reserves, leading to depletion of glycogen and alterations in protein metabolism. Increased proteolysis, reduced protein synthesis, and disruption of enzymatic systems are common responses observed under such conditions. These biochemical changes are often associated with impaired organ function, particularly in metabolically active tissues such as the liver, kidney, and muscle.

In addition, toxic stress can affect enzyme systems involved in metabolism, leading to an imbalance in biochemical homeostasis. Such disruptions may interfere

with growth, reproduction, and survival of aquatic organisms. Over time, these effects can extend beyond individual organisms to influence population dynamics and ecosystem stability. The accumulation of pesticides in aquatic organisms can also result in bioaccumulation and biomagnification, thereby posing potential risks to higher trophic levels, including humans.

Therefore, the present study focuses on evaluating the effects of chlorpyrifos on protein content in different tissues of *Channa punctatus*. By analyzing tissue-specific biochemical changes under sub-lethal and lethal exposures, the study aims to provide insights into the mechanisms of pesticide toxicity. Such information is essential for environmental monitoring, risk assessment, and the development of strategies to minimize the impact of pesticides on aquatic ecosystems and public health.

MATERIALS AND METHODS

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

Five percent homogenates of gill, muscle, and brain tissues and two percent homogenates of liver and kidney were prepared using five percent trichloroacetic acid. The samples were centrifuged at 3000 rpm for 10 minutes, and the supernatant was discarded. The protein residue was dissolved in 1 ml of 1N NaOH. From this extract, 0.2 ml was taken into a test tube and mixed with 5 ml of alkaline copper solution (prepared from 2% sodium carbonate, 0.5% copper sulfate, and 1% sodium potassium tartrate). The mixture was stirred and allowed to stand for 10 minutes. Then, 0.5 ml of Folin phenol reagent (1:1 diluted) was added, and the solution was incubated for 30 minutes for color development. The optical density was measured at 540 nm using a spectrophotometer against a blank. Standard bovine serum albumin (Sigma Chemical Company, USA) was used to prepare the standard curve. The results were expressed as mg/g wet weight of tissue.

RESULTS AND DISCUSSION

The calculated values of total protein content, percentage changes, and standard deviations are presented in Tables I and II.

In control fish (*Channa punctatus*), protein content followed the order:

Kidney > Liver > Brain > Gill > Muscle

This distribution reflects differences in metabolic activity among tissues. The liver, being the center of metabolic regulation and protein synthesis, showed higher protein content.

Effect of Chlorpyrifos Exposure

Under 48-hour exposure to chlorpyrifos (technical grade), protein levels decreased in most tissues.

Order of protein depletion:

- **Lethal exposure:** Liver > Muscle > Kidney > Gill > Brain
- **Sub-lethal exposure:** Liver > Kidney > Muscle > Gill > Brain

This reduction indicates disruption in protein metabolism due to toxic stress.

Table I: 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 48 h.

S. No	Tissue	Control	Sub-lethal	Percent change	Lethal	Percent change
1	Gill	125.42 ±1.19	94.57 ±2.05	24.57	78.64 ±2.23	37.29
2	Brain	133.86 ±1.09	107.56 ±1.17	19.64	88.96 ±1.70	33.54
3	Liver	189.2 ±1.50	126.23 ±2.60	33.28	98.48 ±1.75	47.94
4	Kidney	83.28 ±1.94	58.64 ±3.40	29.58	47.58 ±1.79	42.86
5	Muscle	185.24 ±2.34	130.21 ±3.11	29.70	108.98 ±2.40	41.16

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

Standard Deviation is shown as (±)

The values are significant at $p < 0.05$

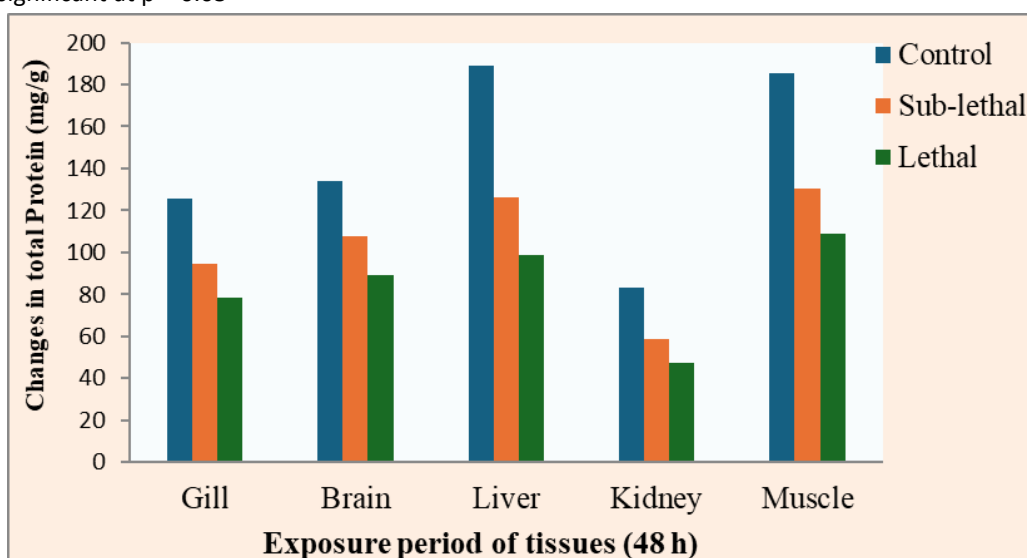


Fig. 1: Changes in the Protein content (mg/gr wet weight of the tissue) in different tissues of *Channa punctatus* on exposure to sub-lethal and lethal concentrations of chlorpyrifos technical grade for 48 h.

Table II: 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 20% EC for 48 h.

S. No	Tissue	Control	Sub-lethal	Percent change	Lethal	Percent change
1	Gill	125.42 ±1.19	98.18 ±1.40	19.21	79.64 ±1.09	34.46
2	Brain	133.86 ±1.09	108.28 ±1.37	16.61	94.53 ±0.12	27.20
3	Liver	189.2 ±1.50	132.78 ±1.38	27.89	112.67 ±2.10	38.81
4	Kidney	83.28 ±1.94	56.18 ±1.36	24.04	48.74 ±2.31	34.09
5	Muscle	185.24 ±2.34	138.62 ±0.86	26.77	118.51 ±1.44	37.39

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

Standard Deviation is shown as (±)

The values are significant at $p < 0.05$

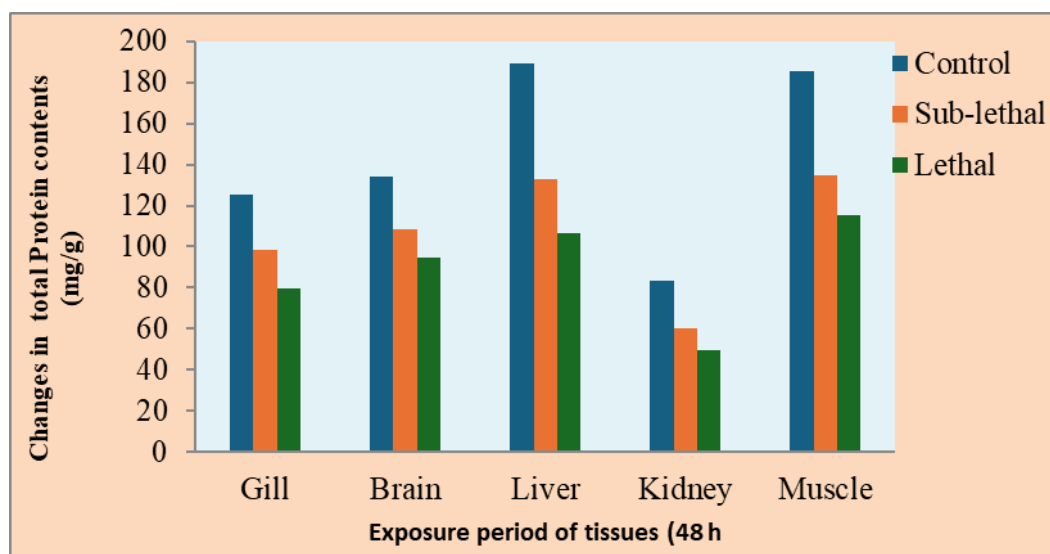


Fig.II: Changes in the Protein content (mg/g wet weight of the tissue) in different tissues of *Channa punctatus* on exposure to sub-lethal and lethal concentrations of chlorpyrifos 20% EC for 48 h.

Protein Changes

A decrease in protein content was observed in all tissues (muscle, brain, gill, kidney, and liver) under both lethal and sub-lethal exposures. This suggests increased energy demand under stress conditions.

The depletion of protein content may be due to:

- Enhanced energy requirements
- Increased proteolysis
- Conversion of proteins into amino acids

Similar reductions in protein levels under pesticide stress have been reported in various aquatic organisms exposed to insecticides such as endosulfan, fenvalerate, monocrotophos, and cypermethrin (Kabeer et al., 1983; Vasanthi and Ramaswamy, 1987; Reddy and Yellamma, 1991).

Studies on fish species such as *Labeo rohita* (Sridevi, 1991; Anuradha, 1993; Veeraiah and Durga Prasad, 1998;

Veeraiah, 2001), *Cyprinus carpio* (Ravisankar et al., 1992), and *Channa punctatus* (Luther Das et al., 1999) also support these observations.

Enzymatic alterations under toxic stress (Helinmayer et al., 1970; Smart, 1978) further contribute to metabolic imbalance, leading to reduced protein levels. The decrease is attributed to enhanced proteolytic activity and impaired protein synthesis (Koundinya, 1979).

Overall, chlorpyrifos exposure causes metabolic stress, disrupting protein metabolism and leading to depletion of protein reserves in fish.

CONCLUSION

Based on the findings of the present study, it can be concluded that chlorpyrifos exposure exerts significant toxic effects on the biochemical composition of various tissues in *Channa punctatus*. In control fish, the protein content was highest in the liver, followed by muscle, kidney, gill, and brain, indicating the metabolic importance of these tissues.

Exposure to both sub-lethal and lethal concentrations of chlorpyrifos for 48 hours resulted in a marked decrease in protein content across all tissues. The liver exhibited the highest degree of depletion, followed by muscle, kidney, gill, and brain, suggesting that metabolically active organs are more susceptible to toxic stress. This reduction in protein levels indicates enhanced proteolysis and possible inhibition of protein synthesis as the organism attempts to cope with increased energy demands under stress conditions.

Similarly, glycogen depletion observed in different tissues reflects increased utilization of energy reserves to meet the metabolic requirements induced by toxic exposure. The combined depletion of proteins and glycogen clearly demonstrates disruption of both protein and carbohydrate metabolism. These biochemical alterations indicate a state of metabolic imbalance and physiological stress in the fish.

Furthermore, the study highlights that even short-term exposure to chlorpyrifos can induce significant biochemical changes, which may impair vital functions such as growth, reproduction, and survival. If such

exposure persists in natural environments, it may lead to long-term ecological consequences, including decline in fish populations and disturbance of aquatic ecosystem balance.

Overall, the findings confirm that chlorpyrifos is highly toxic to aquatic organisms and can significantly alter metabolic processes. Therefore, strict regulation of pesticide usage and continuous monitoring of aquatic environments are essential to prevent ecological damage and protect aquatic biodiversity.

Significance

This study provides valuable insights into the biochemical effects of pesticide contamination in aquatic ecosystems. By demonstrating the impact of chlorpyrifos on protein and glycogen metabolism in *Channa punctatus*, the study emphasizes the role of biochemical parameters as sensitive indicators of toxic stress.

The findings are significant in understanding how pesticides used in agricultural practices can enter aquatic systems through runoff and affect non-target organisms. The observed biochemical changes in fish highlight the potential for bioaccumulation and biomagnification of toxic substances along the food chain, ultimately posing risks to human health.

Additionally, the study contributes to environmental toxicology by providing baseline data that can be used for risk assessment and environmental monitoring programs. It also underscores the importance of adopting sustainable agricultural practices and minimizing excessive pesticide usage.

From an ecological perspective, protecting fish populations is crucial for maintaining the stability of aquatic ecosystems. From a public health perspective, ensuring the safety of aquatic food sources is equally important. Therefore, this study supports the need for stricter environmental regulations, awareness programs, and further research to mitigate the adverse effects of pesticide pollution.

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