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Effects of Chlorpyrifos on Lactate Dehydrogenase Activity in *Channa Punctatus*: A Biochemical Marker of Pesticide-Induced Stress

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Abstract
The present study investigates the biochemical effects of chlorpyrifos-a widely used organophosphate pesticide-on the activity of the key metabolic enzyme Lactate Dehydrogenase (LDH) in various tissues of the freshwater fish *Channa punctatus*. The experiment involved exposure of fish to both sub-lethal and lethal concentrations of chlorpyrifos in two formulations: technical grade and commercial 20% EC. LDH activity was estimated in muscle, liver, kidney, gill, and brain tissues after 24 hours of exposure.
The findings demonstrated a statistically significant increase in LDH activity across all tissues compared to controls, with the liver showing the highest elevation followed by brain and muscle. This enzymatic upregulation suggests a physiological shift towards anaerobic metabolism, potentially as an adaptive response to pesticide-induced hypoxia and mitochondrial dysfunction. Increased LDH activity also indicates cellular damage and compromised membrane integrity, leading to enzyme leakage into the cytoplasm. These responses are consistent with previous reports and highlight the toxic potential of chlorpyrifos on aquatic fauna. Given its rapid response, tissue specificity, and sensitivity to chemical stress, LDH proves to be an effective biomarker for assessing pesticide-induced metabolic disturbances in fish. The study contributes valuable data for environmental monitoring and supports the need for stricter regulations on the use of organophosphate pesticides to safeguard aquatic ecosystems.

Keywords: Lactate dehydrogenase (LDH), chlorpyrifos, channa punctatus, pesticide pollution, environmental stress indicator.

1. Introduction

1.1 Background

Pesticides, especially organophosphates, are extensively used in modern agriculture to control pests and increase crop yields. However, their non-selective toxicity poses serious risks to non-target organisms in aquatic environments, where runoff and leaching often lead to contamination of water bodies. Chlorpyrifos is among the most frequently detected organophosphates in freshwater ecosystems. It is known for its high acute toxicity and potential for bioaccumulation. Once introduced into aquatic systems, chlorpyrifos can interfere with normal physiological and biochemical functions of aquatic fauna, particularly fish.

1.2 Fish as Bioindicators

Fish serve as excellent bioindicators of aquatic pollution due to their sensitivity to contaminants and their position in the food web. Among freshwater species, *Channa punctatus* (spotted snakehead) is a hardy fish commonly used in toxicological studies. Its adaptability to laboratory conditions and its ecological significance in Indian freshwater bodies make it an ideal model organism.

1.3 Role of LDH in Fish Physiology

Lactate dehydrogenase (LDH) is a cytoplasmic enzyme that plays a critical role in glycolysis, particularly under anaerobic conditions. By converting lactate to pyruvate and vice versa, it helps maintain cellular redox balance during oxygen deficiency or increased energy demand. Elevated LDH activity in tissues indicates a shift toward anaerobic metabolism, often associated with stress responses to toxicants. Therefore, LDH activity is a widely accepted biochemical marker for cellular stress, hypoxia, and tissue damage. Studying tissue-specific alterations in LDH levels can provide insights into the physiological disruptions caused by environmental contaminants like chlorpyrifos.

2. Review of Literature

Several researchers have explored the enzymatic responses of fish under pesticide exposure:

- Verma and Gupta (1976) noted a reduction in LDH activity in the liver, kidney, and intestine of *Channa punctatus* exposed to diazinon, while the brain showed an increase, highlighting tissue-specific responses.
- Joshi (1978) and Verma *et al*. (1982a) reported decreased transaminase (GOT and GPT) activity in liver and muscle of *Cyprinus carpio* under fenthion exposure, attributed to enzyme leakage and tissue damage.
- Amanullah *et al*. (2010) observed elevated LDH and reduced acetylcholinesterase (AChE) in *Lamellidens marginalis* following chlorpyrifos exposure, indicating both neurotoxic and metabolic stress.
- Natarajan (1984) and Ghosh (1987) reported similar LDH elevations in response to different organophosphates, supporting its role as a consistent biomarker.
- Fluke (1972) associated elevated LDH with increased membrane permeability and cellular necrosis.

  These studies provide strong evidence for LDH as a stress biomarker under chemical toxicity and validate the present study's focus.

3. Materials and Methods

3.1 Animal Collection and Acclimatization

Channa punctatus specimens (25–30 g) were collected from local freshwater bodies. The fish were acclimated in glass aquaria for 10 days under laboratory conditions (25±2°C, 12 h light/dark cycle). They were fed ad libitum and fasted 24 h prior to the experiment.

3.2 Dose Selection

Sub-lethal and lethal doses of chlorpyrifos (both technical and 20% EC) were determined via range-finding tests. The selected doses were based on 1/10th and full 24-hour LC50 values, respectively.

3.3 Experimental Setup

Fish were randomly divided into five groups (n = 5 each):

- Control (no exposure)
- Sub-lethal Technical Grade
- Lethal Technical Grade
- Sub-lethal 20% EC
- Lethal 20% EC

Each group was maintained in a separate tank for 24 hours, with careful monitoring.

3.4 Sample Collection

Post-exposure, fish were euthanized, and tissues (gill, brain, liver, kidney, and muscle) were dissected, weighed, and homogenized in 0.25 M ice-cold sucrose (2% w/v). Homogenates were centrifuged at 1000 rpm for 15 minutes, and supernatants were used for LDH assay.

3.5 Enzyme Assay Procedure

Following Srikanthan and Krishna Murthy (1955), LDH activity was measured via colorimetric analysis using INT and NAD as substrates.

Reaction Mixture

- 0.5 ml lithium lactate
- 0.5 ml phosphate buffer
- 0.2 ml NAD
- 0.2 ml INT
- 0.6 ml supernatant

The mixture was incubated at 37°C for 30 minutes. Reactions were stopped with 5ml acetic acid. A zero-time control was included. Formazan formed was extracted overnight in 5 ml cold toluene, and absorbance measured at 495 nm. Results were calculated as µmoles of formazan/mg protein/hour.

3.6 Protein Estimation

Protein content was determined by Lowry's method for normalization of LDH activity.

3.7 Statistical Analysis

Data were analyzed using one-way ANOVA followed by post-hoc Tukey's test. Values were expressed as mean ± SD. A p-value < 0.05 was considered statistically significant.

4. Results

4.1 LDH Activity Trends

Both formulations of chlorpyrifos caused significant elevation in LDH activity in all tissues. The liver consistently showed the highest percent increase, followed by brain and muscle.

4.2 Comparison: Technical Grade vs. 20% EC

Technical grade chlorpyrifos showed slightly higher LDH induction than 20% EC in most tissues, suggesting higher toxicity or bioavailability.

Key Patterns Observed

- Liver and brain exhibited the greatest sensitivity.
- Gill and kidney had moderate LDH increases, indicating respiratory and excretory stress.
- Muscle showed elevated activity, possibly reflecting increased energy demand under stress.

4.3 Statistical Summary

All enzyme activity increases were statistically significant ($p < 0.05$), with lethal doses inducing higher responses than sub-lethal ones. The enzyme LDH activity levels of muscle, brain, liver, gill and kidney of control fish were almost constant. The values of control LDH activity in test tissues of the fish *Channa punctatus* were in the order of:

Technical grade

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

20% EC

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

In sub-lethal exposure of chlorpyrifos technical grade, the LDH enzyme activity was observed to elevate in almost all the tissues of fish *Channa punctatus.* The percent changes in the LDH activity levels, of the test fish appeared to be in the order of:

24 h Technical grade

Lethal: Liver > Brain > Muscle > Kidney > Gill

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

24 h 20% EC

Lethal: Liver > Brain > Kidney > Muscle > Gill

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

The activity of LDH was highly elevated in the present study, it was noticed that under exposure to chlorpyrifos indicating increase in the anaerobic respiration to combat the demand of energy while the aerobic oxidation is reduced. Lactate dehydrogenase (LDH) transforms lactate to pyruvate and plays key role in metabolism of carbohydrates.

The increase of LDH activity during conditions favoring anaerobic respiration to meet the energy demands lowers the aerobic respiration (Martin *et al*., 1983) [8]. The earlier reports on *Tilapia mossambica* (Anastasi and Bamistor, 1980;

Table 1.1: Changes in the specific activity levels of LDH (µ moles of formazane/mg Protein/h) in different tissues of *Channa punctatus on* exposure to sub-lethal and lethal concentrations of chlorpyrifos technical grade for 24 h.

S. No	Tissue	Control	Sub-lethal	Percent change	Lethal	Percent change
1	Gill	0.1118±0.09	0.1352±0.006	11.80	0.1424±0.025	27.37
2	Brain	0.0303±0.002	0.0366±0.001	20.79	0.0385±0.002	27.06
3	Liver	0.1015±0.006	0.1285±0.025	26.60	0.1471±0.062	44.92
4	Kidney	0.0926±0.001	0.1168±0.09	21.81	0.1209±0.006	30.56
5	Muscle	0.1413±0.027	0.1693±0.013	19.81	0.1886±0.09	32.76

The values of the results are the mean values of five observations
Standard Deviation is shown as (±)
The values are significant at $p<0.05$

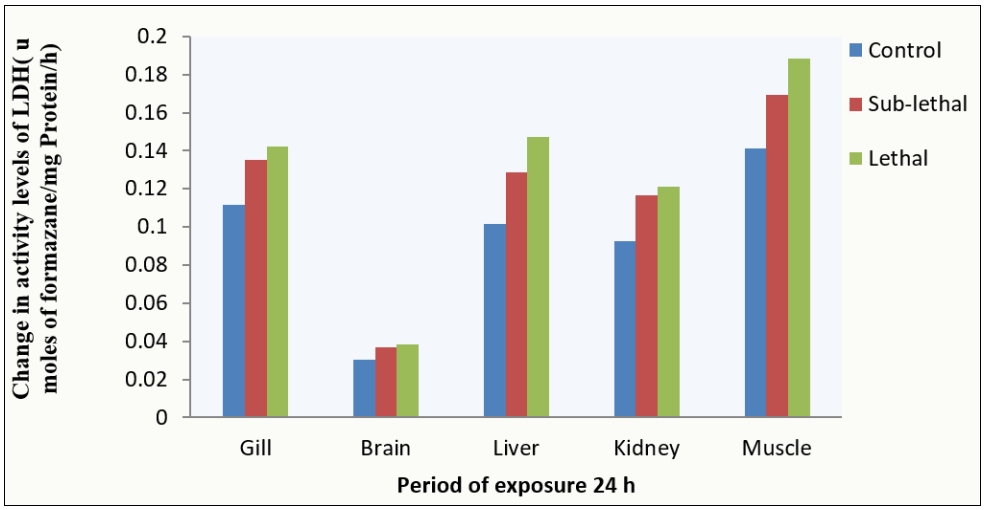

Fig 1.1: Changes in specific activity levels of LDH (µ moles of formazane/mg Protein/h) in different tissues of *Channa punctatus on* exposure to sub-lethal and lethal concentrations of chlorpyrifos technical grade for 24 h.

Table 1.2: Changes in the specific activity levels of LDH (µ moles of formazane/mg Protein/h) in different tissues of *Channa punctatus on* exposure to sub-lethal and lethal concentrations of chlorpyrifos 20% EC for 24 h.

S. No.	Tissue	Control	Sub-lethal	Percent change	Lethal	Percent change
1	Gill	0.1118±0.09	0.1268±0.002	13.41	0.1392±0.025	24.50
2	Brain	0.0303±0.002	0.0362±0.005	19.47	0.0398±0.006	31.35
3	Liver	0.1015±0.006	0.1242±0.027	22.36	0.1392±0.002	37.14
4	Kidney	0.0956±0.001	0.1142±0.003	19.45	0.1218±0.013	27.40
5	Muscle	0.1413±0.027	0.1626±0.002	15.07	0.1782±0.072	26.11

The values of the results are the mean values of 5 observations
Standard Deviation is shown as (±)
The values are significant at $p<0.05$

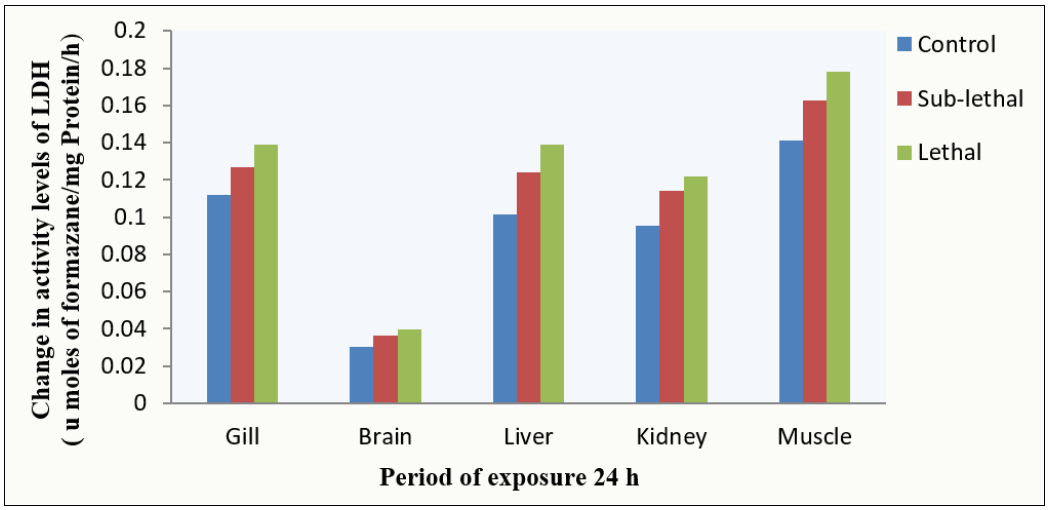

Fig 1.2: Changes in the specific activity levels of LDH (µ moles of formazane/mg Protein/h) in different tissues of *Channa punctatus on* exposure to sub-lethal and lethal concentrations of chlorpyrifos 20% EC for 24 h.

5. Discussion

The elevation in LDH activity confirms the metabolic shift from aerobic to anaerobic respiration under pesticide-induced stress. Chlorpyrifos interferes with cellular oxygen utilization and mitochondrial function, compelling the organism to rely on anaerobic glycolysis.

The liver's central role in detoxification explains its high LDH response. Increased activity in the brain indicates neuro-metabolic disruption, consistent with known neurotoxicity of organophosphates. Muscle tissue shows metabolic compensation for energy under stress, while elevated LDH in gills and kidneys indicates functional stress in respiration and excretion.

These findings align with existing literature and strengthen the case for LDH as a key biomarker of aquatic toxicity.

Further insight into the elevated LDH activity in *Channa punctatus* reveals its dual implication: first, as a biochemical compensatory response to sustain energy production when oxidative phosphorylation is compromised, and second, as a consequence of cytotoxic effects leading to membrane damage and leakage of cytosolic enzymes. In fish, such a shift towards anaerobic metabolism often reflects physiological strain, where the oxygen transport mechanisms, primarily gill function, are impaired by the toxicant, reducing the capacity for efficient aerobic respiration.

The variations in LDH response across tissues may also relate to the metabolic demands and sensitivity of each organ. For instance, the liver plays a central role in biotransformation and detoxification, explaining its high susceptibility. Similarly, increased LDH in the brain suggests neurotoxic implications of chlorpyrifos exposure, possibly linked with disturbed ion gradients or compromised neuronal signaling pathways beyond AChE inhibition.

The modulation of LDH activity could also be linked to secondary oxidative stress pathways. Chlorpyrifos exposure is known to generate reactive oxygen species (ROS), which can oxidize lipids, proteins, and nucleic acids, leading to cellular dysfunction. The shift to anaerobic glycolysis and subsequent LDH elevation may thus serve as an adaptive strategy to circumvent mitochondrial injury.

6. Ecotoxicological Significance

This study underscores the ecological risk of chlorpyrifos in freshwater systems. Elevated LDH activity reflects underlying metabolic disturbances that, if sustained, can impair growth, reproduction, and survival in aquatic species.

Monitoring LDH activity could serve as an early warning system in environmental risk assessments. The results are pertinent not only to ecotoxicologists but also to environmental managers and policymakers.

The use of LDH as a biomarker has practical advantages in environmental monitoring programs. Unlike many other markers, LDH is relatively easy to assay, responds quickly to stressors, and can be monitored non-lethally in some species. These features make it suitable for early detection of sub-lethal pollution levels, allowing proactive mitigation before irreversible ecological damage occurs.

In aquatic environments where multiple pollutants may coexist, LDH responses can also be used alongside other markers like acetylcholinesterase (AChE), glutathione-S-transferase (GST), catalase (CAT), and superoxide dismutase (SOD) to construct a multi-biomarker index (MBI). Such indices provide a more holistic assessment of ecosystem health.

Furthermore, regulatory agencies can integrate LDH response profiles into ecological risk assessments for pesticides. Coupled with data from field studies and mesocosm experiments, such laboratory-based enzymatic profiles can help identify sensitive species and critical exposure thresholds, informing water quality guidelines.

Mechanistic Considerations

Recent molecular studies suggest that chlorpyrifos-induced LDH alterations may be mediated by transcriptional regulation of metabolic genes. For instance, hypoxia-inducible factor 1-alpha (HIF-1α), a key regulator of anaerobic metabolism, may be upregulated under chlorpyrifos stress, enhancing LDH gene expression. Verification through gene expression studies (e.g., RT-qPCR) could confirm this regulatory pathway.

Epigenetic mechanisms are also emerging as potential modulators of toxicity responses. DNA methylation changes and histone modifications under chlorpyrifos exposure could influence LDH expression and other metabolic enzymes, contributing to long-term physiological alterations in exposed populations.

Conclusion

Exposure to chlorpyrifos, even at sub-lethal levels, significantly affects LDH activity in various tissues of *Channa punctatus*. This enzymatic alteration indicates cellular stress and a compensatory shift to anaerobic metabolism. LDH can be used as a sensitive biomarker for detecting pesticide-induced biochemical stress in aquatic organisms. Effective monitoring and regulation of pesticide use are imperative to safeguard aquatic life and ecosystem health. The consistent elevation of LDH activity in multiple tissues of *Channa punctatus* underscores the enzyme's diagnostic potential in detecting pesticide-induced metabolic disruption. These findings strengthen the case for adopting LDH as a routine biomarker in ecotoxicology and call for expanded studies involving time-series exposures, chronic low-dose studies, and recovery monitoring. Given that aquatic ecosystems are complex and often exposed to multiple pollutants simultaneously, future investigations should consider interactions between chlorpyrifos and other contaminants. Understanding additive or synergistic effects will be vital in shaping effective environmental protection strategies.

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