The Toxicity Effect of Chlorpyrifos on the Histopathology of Hepatopancreas of Freshwater crab *Barytelphusa Cunicularis*

Dr. Tasneem Jahan[1], Asima Begum Afreen[2], Maliha Afshan[3], Mahveen Jahan[4], Shaikh Shainaz Abdullah[5]

[1]Associate Professor, Department of Zoology, St. Ann's College for Women, Hyderabad, Telangana, INDIA,
Email: tasneem.jahan2024@gmail.com

[2]Assistant professor Department of Zoology, Anwarul Uloom College, Hyderabad, Telangana, INDIA,
[3]Assistant professor Department of Zoology, Anwarul Uloom College, Hyderabad, Telangana, INDIA,
[4]Assistant professor Department of Zoology, Anwarul Uloom College, Hyderabad, Telangana, INDIA,
[5]Assistant professor, Department of Zoology, St. Ann's Junior College for Women, Hyderabad, Telangana, INDIA.

ABSTRACT

Chlorpyrifos, an organophosphate insecticide, is widely used in agriculture but poses significant ecological risks to non-target organisms, including aquatic species. This study investigates the toxicity of chlorpyrifos on the histopathology of the hepatopancreas in the freshwater crab *Barytelphusa cunicularis*, a species commonly found in aquatic ecosystems. The hepatopancreas, a vital organ involved in digestion and detoxification, was selected to evaluate the cellular and tissue-level impacts of chlorpyrifos exposure. Crabs were exposed to varying concentrations of chlorpyrifos, and the hepatopancreatic tissues were examined histopathologically after a specified period of exposure. The results revealed significant histopathological alterations, including cellular degeneration, necrosis, vacuolization, and inflammatory responses in the hepatopancreas. These findings suggest that chlorpyrifos induces considerable damage to the hepatopancreatic tissue, compromising its physiological functions. The study underscores the potential ecological threat posed by chlorpyrifos to freshwater ecosystems, particularly affecting species like *Barytelphusa cunicularis*, and calls for further research into the long-term environmental and health implications of chlorpyrifos contamination.

Keywords: Chlorpyrifos, Insecticide, Toxicity, *Barytelphusa cunicularis*, Histopathology.

Introduction

Biochemical studies play a crucial role in understanding the physiological and molecular responses of organisms to various environmental stressors, including pollutants like pesticides. One such pesticide, chlorpyrifos, is widely used in agriculture to control a variety of pests. However, its toxic effects on non-target organisms, particularly aquatic species, have raised concerns regarding its ecological impact. (Rao & Reddy, 2003)

Barytelphusa cunicularis, a species of freshwater crab, serves as an excellent model organism to study the effects of environmental contaminants such as chlorpyrifos. These crabs are crucial to aquatic ecosystems, as they play an important role in the food web and nutrient cycling. However, exposure to chemicals like chlorpyrifos can disrupt their biochemical processes, potentially leading to detrimental effects on their survival, reproduction, and overall health. (Mackie & Bischof, 2014)

Chlorpyrifos is an organophosphorus compound that works by inhibiting acetylcholinesterase, an enzyme crucial for proper nerve function. Its accumulation in aquatic environments, due to runoff or direct application, can lead to toxic concentrations that affect organisms in the ecosystem. In

crabs like *Barytelphusa cunicularis* , this can manifest as changes in biochemical parameters such as enzyme activity, oxidative stress markers, and metabolic shifts, which can impact their health and behaviour.(Singh & Soni, 2009)

The objective of biochemical studies on *Barytelphusa cunicularis* in the context of chlorpyrifos exposure is to assess the physiological impacts of the pesticide and identify biomarkers of toxicity. These studies are essential for understanding the broader implications of pesticide use on freshwater biodiversity and can help inform regulations to minimize environmental harm from chemical pollutants. (Gauthier et al., 2014)

Water bodies are continuously being contaminated by agricultural pesticide and affect the non-target organism. Pollution of aquatic fauna by any pesticide can have deleterious effect on aquatic organisms. The nature of the effect may varies and may cause structural and functional modification at both, cellular and sub cellular levels in organisms.These toxicants find their way into the body of aquatic organism by route of gills, oral membranes, gastrointestinal mucosa and general body surface (Baskaran & Arumugam, 2008).

The toxicant after getting entered into the body of an aquatic organism reaches to different tissue, gets accumulated their and finally causes

considerable damages; which seriously affect the physiological function of tissue concerned. The cellular damage and resulting disease can be detected by the histopathological studies. Histopathological studies provided a clear picture of the toxicant stress-induced damages in the cellular organization. (Singh & Dubey, 2005).

In crustacean hepatopancreas is the important tissues. The mobilization of lipid content and osmoregulation are mainly controlled by tissue of these two organs. Abundant enzyme are commonly present in hepatopancreas of crustaceans, which break down carbohydrates and related compounds. Even slight damage in to the cells of hepatopancreas can cause hazardous effect on the physiology of digestion.

Barytelphusa cunicularis is key species having good nutritional value for consumption. They have very good demand in markets, but day by day there population is decreasing, because of adverse effect of pesticides. Therefore present work has been undertaken to study the impact of chlorpyrifos pesticide, on the hepatopancreas of fresh water crab *Barytelphusa cunicularis*. (Maiti, 2010).

MATERIALS AND METHODS

Freshwater crab *Barytelphusa cunicularis* , Chlorphyriphos pesticide (20%) , Folin’s reagent (90%) , distilled water.

Fig.1- Chlorpyrifos pesticide *Fig.2 - Barytelphusa cunicularis*

Fig.3 - Barytelphusa cunicularis

Experimental Setup

Crab Exposure: Six crabs were placed individually in three separate set-ups. The crabs were exposed to the concentrations given below of chlorpyrifos for 12 hours:

Set-up 1: 0.1 ml of chlorpyrifos in 500ml of water

Set-up 2: 0.2 ml of chlorpyrifos in 500ml of water

Set-up 3: 0.3 ml of chlorpyrifos in 500ml of water

The Hepatopancreas were dissected and they were stored in 90% formlain for further experiment.

Fig.4 - Selecting the crabs for exposure of the pesticide.

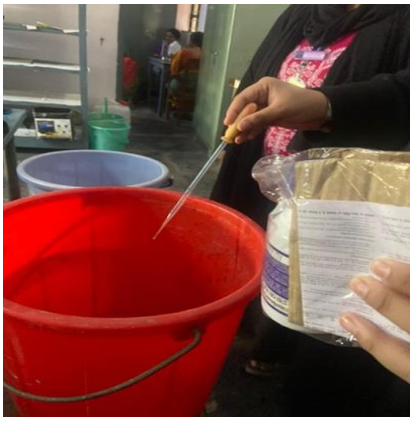

Fig.5 - Addition of the chlorpyrifos pesticide.

Behavioral Observation: The crabs were observed for 12 hours to assess the behavioral changes in response to the chlorpyrifos exposure.

In set-up 1,the crabs with 0.1ml concentration of the pesticide displayed significant inactivity.

In the set-up 2, the crabs exposed to a 0.2ml concentration of the pesticide exhibited marked inactivity and lethargy.

In the set-up 3, the crabs exposed to higher concentrations of the pesticide, such as0.3 ml, exhibited toxic aggression and a strong inclination to escape the water.

Tissue Collection: After the 12-hours exposure period, the crabs were dissected, and the hepatopancreas (a vital digestive and metabolic organ in crabs) was dissected and preserved in 90% formalin for further analysis to histopathology lab.

Histological Studies: Microscopic examination of the hepatopancreas tissue will be conducted to assess any structural damage or abnormalities caused by chlorpyrifos exposure.

Microtomy

Microtomy is the means by which tissue can be sectioned and attached to a surface for further microscopic examination. Most microtomy is performed on paraffin-embedded tissue blocks. The basic instrument used in microtomy is the microtome; an advancing mechanism moves the object (paraffin block) for a predetermined distance until it is in contact with the cutting tool (knife or blade). The specimen moves vertically past the cutting surface producing a tissue section. Good technique is mastered through continuous practice. (Lena T. Spencer, John D. Bancroft; 2008).

Procedure: The procedure for preparing a histopathology slide of a crab involves fixing, dehydrating, clearing, embedding, sectioning, staining, and mounting.

Steps:

- Fixation: Use a formalin solution to preserve the tissue structure and prevent degradation
- Dehydration: Use ethanol to remove water from the tissue
- Clearing: Use xylene to remove ethanol and allow paraffin to infiltrate the tissue
- Embedding: Embed the tissue in paraffin wax to create a block
- Sectioning: Use a microtome to cut thin sections of the tissue
- Staining: Use a stain to make the tissue components visible
- Mounting: Transfer the tissue sections to glass slides
- Additional information
- The tissue block is supported by the solid paraffin wax, which allows for thin sectioning
- The tissue sections are cut to a thickness of about 4-5 micrometers, which is the optimal thickness for staining
- The appropriate stain is chosen based on its unique properties
- For example, Hematoxylin and Eosin are combined in H&E staining

Controlled crab

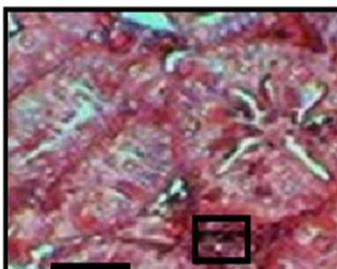

Fig.6- Hepatopancreas of *Barytelphusa cunicularis* *Fig.8* - *Hepatopancreas appearing in healthy normal condition*

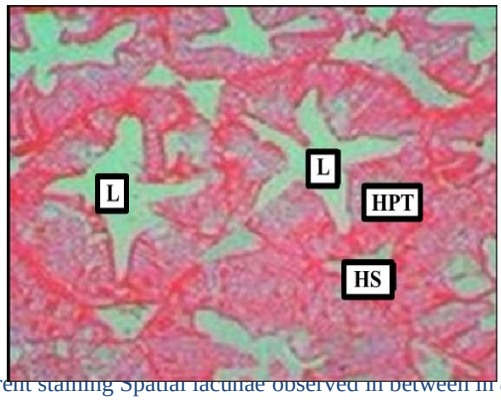

Fig.9 - Hepatopancreas appearing in healthy normal condition in different staining Spatial lacunae observed in between in *areas*

0.1 ml Concentration

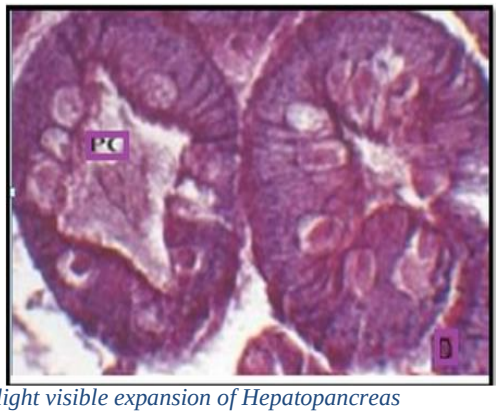

Fig.9 - Slight visible expansion of Hepatopancreas

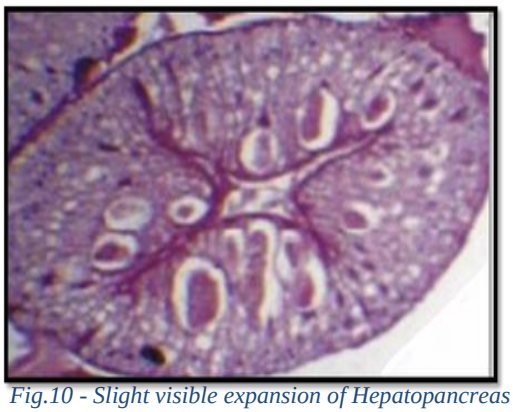

Fig.10 - Slight visible expansion of Hepatopancreas

0.2 ml Concentration

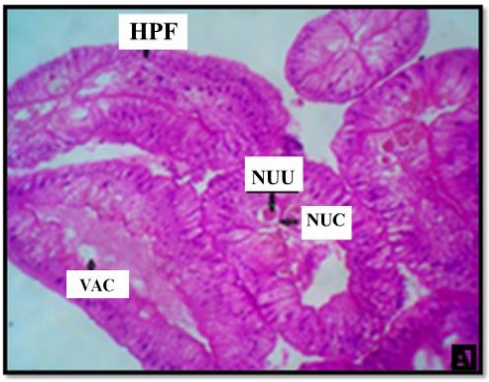

Fig.11 - Hepatopancreas Large vacuoles, expanded lumen, seen in the hepatopancreatic cells.

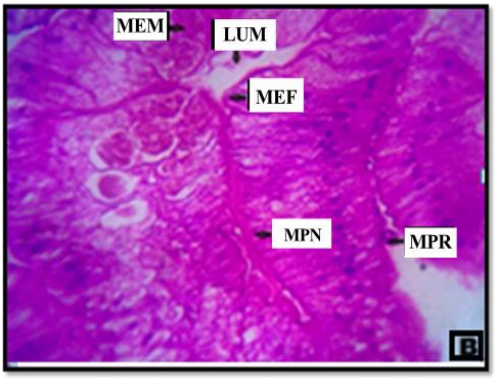

Fig.12 - Hepatopancreas widespread degeneration of the tubular and intratubular tissues are most evident in the hepatopancreas cells.

0.3 ml concentration

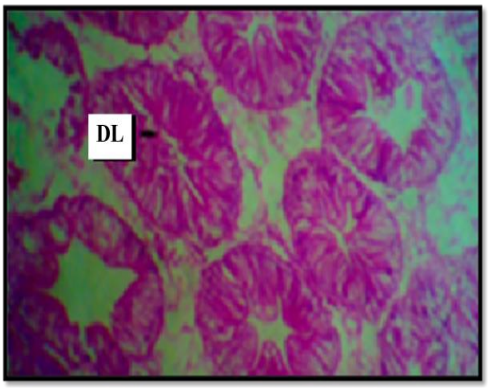

Fig.13 - Hepatopancreas- Distended lumen

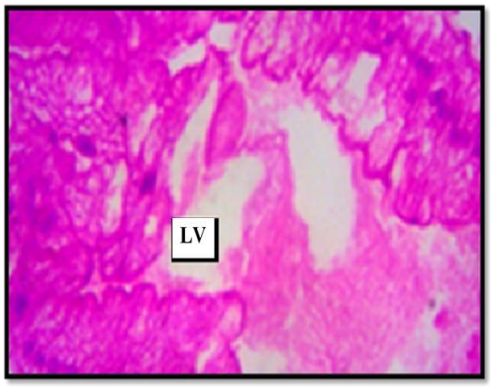

Fig.14 - Hepatopancreas - Formation of large vacuoles

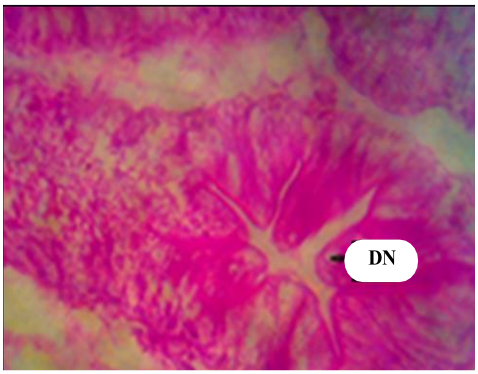

Fig.15 - Hepatopancreas - the nuclei entirely absent.

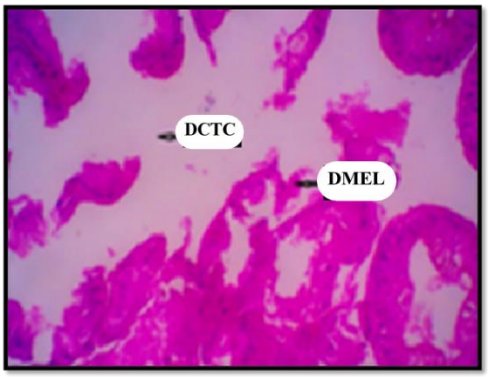

Figure 1Fig.16- The muscle bundles are completely destroyed, with the striations discontinuous, damaged connective tissue layer and damaged myoepithelial layer.

Observations and Potential Reasons for Chlorpy rifos Effects on Crab

Hepatopancreas:

- **Control Crab (No Chlorpyrifos):**

Observation: Hepatopancreas appears healthy and normal, with spatial lacunae (spaces) observed.

Reason: This represents the baseline, indicating the natural structure and function of the hepatopancreas in a healthy crab. The spatial lacunae are likely normal anatomical features.

- **0.1 ml Chlorpyrifos Concentration:**

Observation: Slight visible expansion of the hepatopancreas.

Reason: This initial expansion could be an early inflammatory response to the low concentration of chlorpyrifos. The hepatopancreas is likely reacting to the toxicant by increasing fluid volume, potentially to dilute or neutralize it. It may also be the start of cellular swelling due to disruption of ion pumps within the cells.

- **0.2 ml Chlorpyrifos Concentration**:

Observation:

* Large vacuoles within the hepatopancreas cells.

* Expanded lumen (the internal space of the tubules).

* Widespread degeneration of tubular and intratubular tissues.

Reason:

* Vacuoles: These are likely formed due to cellular stress and disruption of cellular processes. Chlorpyrifos can interfere with enzyme activity and membrane integrity, leading to the accumulation of fluids and cellular debris within vacuoles.

* Expanded Lumen: This could be due to increased fluid accumulation and cellular damage, causing the tubules to swell.

* Degeneration: Chlorpyrifos is an organophosphate insecticide that inhibits acetylcholinesterase, disrupting nerve function. This can lead to cellular damage and death, manifesting as tissue degeneration. Additionally, chlorpyrifos can cause oxidative stress, which further damages cellular components.

- **0.3 ml Chlorpyrifos Concentration:**

Observation:

* Complete destruction of muscle bundles.

* Discontinuous muscle striations.

* Absence of nuclei.

* Vacuole formation.

Reason:

* Muscle Bundle Destruction & Discontinuous Striations: The high concentration of chlorpyrifos has caused severe damage to the muscle tissue, indicating a breakdown of cellular structure and function. The loss of striations indicates the destruction of the sarcomeres, the contractile units of muscle.

* Absence of Nuclei: This signifies cell death (necrosis). The nuclei, which contain the cell's DNA, are no longer present, indicating irreversible damage.

* Vacuole Formation: This reinforces the ongoing cellular degeneration and accumulation of cellular debris. The vast amount of vacuoles, compared to the lower dose, shows the increased amount of cellular breakdown.

- **Overall**: At this concentration, the hepatopancreas has suffered extensive cellular damage, indicating severe toxicity.

DISCUSSION:

Barytelphusa cumicularis is a species of freshwater crab found in India, and it serves as an important organism for studying the effects of pollutants like pesticides on aquatic life. The hepatopancreas is a vital organ in crustaceans, playing a significant role in digestion, nutrient storage, and detoxification. In this context, chlorpyrifos, a commonly used organophosphate pesticide, has been studied for its

impact on the hepatopancreas and overall health of these crabs.(Rajkumar & Sridhar, 2013).

When *Barytelphusa cunnicularis* is exposed to chlorpyrifos, several physiological and biochemical changes occurs, particularly in the hepatopancreas. The hepatopancreas, similar to the liver in vertebrates, is responsible for the detoxification of harmful substances, including pesticides.

Following are the changes due to chlorpyrifos exposure:

- Chlorpyrifos impairs the function of detoxification enzymes in the hepatopancreas.

  This could reduce the crab's ability to metabolize and detoxify the pesticide, leading to accumulation and exacerbating toxic effects.

- Chronic exposure to chlorpyrifos could lead to significant histopathological changes in the hepatopancreas, such as:

1. Degeneration of cells: Cellular structures in the hepatopancreas may show signs of damage or necrosis.
2. Inflammation: Inflammatory responses could be triggered as the organism attempts to respond to the toxin. (Mantovani & Mazzarino, 2015).

- Since the hepatopancreas is responsible for the digestion and

absorption of nutrients, any impairment of its function can lead to malnutrition and reduced overall health. This could affect the growth and reproductive capacity of the crab, making it more vulnerable to other environmental stressors.

Moreover, chronic exposure to chlorpyrifos may result in structural changes in the hepatopancreas. Histological studies have shown that organophosphate exposure can lead to alterations such as vacuolation, tissue degeneration, and inflammation in the hepatopancreatic cells. These changes impair the organ's ability to function properly, affecting both digestion and the breakdown of harmful substances.(Gupta & Sharma, 2017).

In addition to oxidative damage, chlorpyrifos can also interfere with the hormonal regulation of the hepatopancreas. Organophosphates have been shown to alter endocrine functions in crustaceans, which can further affect the liver-pancreas organ's ability to regulate metabolism and energy storage. This could lead to an overall weakening of the organism's health, reducing its ability to survive in contaminated environments.(Dutta et al., 2008).

Given the central role of the hepatopancreas in the detoxification and metabolic processes of *Barytelphusa cunicularis*, exposure to chlorpyrifos

represents a significant threat not only to individual organisms but also to the broader ecosystem. (Kumar & Yadav, 2017).

Deltamethrin is a type of pyrethroid insecticide widely used to control pests, acting by disrupting the nervous system of insects and causing paralysis and death. However, its toxicity also affects non-target organisms, including freshwater species like *Barytelphusa cunicularis*, a type of freshwater crab. When exposed to deltamethrin, these crabs may experience a range of harmful effects, especially in aquatic environments where the chemical can accumulate in the water.(Al-Badran et al., 2019).

The toxicity of deltamethrin to *Barytelphusa cunicularis* is most evident in its lethal effects.(Masud & Singh, 2011). Pyrethroids, including deltamethrin, cause the disruption of sodium channels in the nerve cells, leading to persistent depolarization and neuronal hyperactivity.(Rehman et al., 2014). This results in paralysis, impaired locomotion, and ultimately mortality in affected individuals. In addition to mortality, exposure to sub-lethal concentrations has been shown to alter the behavior of the crabs, manifesting as reduced activity levels, erratic swimming, and an inability to respond appropriately to environmental stimuli. (Sharma et al., 2015).

Acute toxicity coragen (chlorantraniliprole) on *Barytelphusa cunicularis*

manifest as paralysis, impaired locomotion, and mortality due to the chemical's action on the nervous system.(Lu et al., 2019).

Even sub-lethal concentrations may induce behavioral changes, such as erratic swimming, reduced activity, and impaired feeding behavior, which could compromise survival by affecting foraging efficiency and predator avoidance.(McLuckie et al., 2020).

Moreover, chronic exposure to lower concentrations of chlorantraniliprole may lead to sub-lethal physiological effects, including reduced reproductive success, growth inhibition, and disruptions to the molting process, potentially impacting the population dynamics of the species. (Gebauer et al., 2017).

Bioaccumulation of chlorantraniliprole in the tissues of *Barytelphusa cunicularis* is a concern, particularly in environments with persistent pesticide exposure. Over time, this bioaccumulation could lead to chronic toxicity, affecting the long-term health and fitness of the species.(Wacker & Harzsch, 2021).

CONCLUSION

The present study highlights the significant impact of chlorpyrifos exposure on the hepatopancreas of *Barytelphusa cumicularis*, demonstrating its potential to disrupt crucial physiological and

biochemical functions. The impairment of detoxification enzymes, coupled with histopathological changes such as cellular degeneration and inflammation, indicates severe toxicity that compromises the crab's overall health. Which results in malnutrition and weakened physiological state further suggest that chronic exposure could have long-term consequences on growth, reproduction, and survival.

These findings emphasizes the ecological risks associated with organophosphate present in pesticides like chloropyrifos in freshwater ecosystems and focuses the need for strict regulations and sustainable pest management practices to protect aquatic biodiversity.

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