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Toxic Impact of Flubendiamide on Hepatic Tissue Architecture of Freshwater Fish *Labeo rohita*

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Abstract: ***Flubendiamide, a widely used diamide insecticide, is increasingly detected in aquatic ecosystems due to agricultural runoff, posing risks to non-target organisms such as fish. The present study investigates the acute effects of flubendiamide on liver histopathology in Labeo rohita. Fingerlings were divided into three experimental groups Control, LC_0, and LC_{50} and exposed to the predetermined concentration of flubendiamide for 96 hrs under controlled laboratory conditions. Histological evaluation revealed several pathological alterations, including hepatocyte necrosis, vacuolation, sinusoidal dilation, nuclear pyknosis, blood congestion, and disruption of hepatic cords in LC_0, and LC_{50} group. Tissue degeneration increased with concentration, indicating dose-dependent hepatotoxicity. These findings demonstrate that flubendiamide induces substantial structural damage to hepatic tissue, compromising metabolic and detoxification functions in fish. The study highlights the ecological risks associated with indiscriminate pesticide use and underscores the need for stringent monitoring of agricultural runoff into aquatic habitats.***

Keywords: Labeo rohita; Flubendiamide; Liver histopathology; Hepatotoxicity

I. INTRODUCTION

Pesticides represent one of the most hazardous chemical pollutants introduced into the environment. Although their usage has greatly supported agricultural productivity and contributed to human welfare, excessive and indiscriminate application has raised serious concerns regarding their detrimental effects on non-target organisms. Among these, fish occupy an important ecological position and serve as a vital source of nutrition for humans [1] [2] [3]. Modern agricultural practices rely heavily on agrochemicals including fertilizers, insecticides, herbicides, and fungicides to maximize crop production; however, continuous and unregulated use of these compounds results in contamination of nearby aquatic environments such as ponds, lakes, rivers, and reservoirs through surface runoff, direct discharge, and leaching. This contamination progressively deteriorates water quality and disrupts aquatic ecosystems [4] [5].

Once released into freshwater habitats, pesticides exhibit toxic effects due to their chemical persistence, lipophilic nature, bioaccumulation potential, and tendency for biomagnification across trophic levels, ultimately threatening ecosystem stability [6]. Fish readily absorb these toxicants through their gills, skin, and digestive tract, making them highly susceptible to chemical stress and valuable bioindicators of environmental health. Exposure to pesticides may interfere with essential physiological and biochemical processes, leading to impaired growth, reproductive dysfunction, behavioral abnormalities, suppressed immunity, and even mortality.

The liver is a key organ involved in metabolic regulation and detoxification of xenobiotics in fish. Due to its central role in biotransformation, the liver is often the first organ to exhibit toxic damage when exposed to contaminants. Histopathological analysis of liver tissue is therefore considered a sensitive and reliable method for evaluating the impact of toxicants and assessing overall fish health.

Flubendiamide, a modern insecticide belonging to the phthalic acid diamide group, acts by selectively activating insect ryanodine receptors (RyRs), causing uncontrolled release of intracellular Ca^{2+}, sustained muscle contraction, and paralysis of target pests [7] [8] [9]. Although designed for selective action against lepidopterans, recent findings suggest that flubendiamide may also disrupt calcium-mediated physiological processes in non-target aquatic organisms, including fish. Such interference can lead to oxidative stress, metabolic dysregulation, neurotoxicity, and structural tissue injury. Despite widespread agricultural application of flubendiamide, scientific information regarding its potential hepatotoxic effects on freshwater species remains limited. Therefore, the present study aims to evaluate the histopathological alterations in the liver of *Labeo rohita* following acute exposure to flubendiamide, providing insight into its toxic impact and potential ecological risk.

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II. MATERIALS AND METHODS

A. Experimental Fish and Chemicals

Healthy fingerlings of the freshwater fish *Labeo rohita* were procured from a fish seed rearing center located at Kale, Kolhapur, Maharashtra. Upon arrival in the laboratory, the fish were disinfected by briefly immersing them in a 0.1% potassium permanganate ($KMnO_4$) solution to remove external parasites and surface microbial contaminants. The fish were subsequently acclimatized for 15 days in well-aerated glass aquaria filled with dechlorinated tap water. During the acclimation period, fish were fed daily with a commercial floating pelleted feed. To maintain optimal water quality, uneaten feed and fecal matter were removed from the aquaria through gentle siphoning every day. The physicochemical characteristics of the water, including pH, temperature, dissolved oxygen, hardness, and alkalinity, were regularly monitored and maintained within suitable ranges for *L. rohita.* All parameters were analyzed according to standard procedures described by the American Public Health Association (APHA, 1998) [10]. Commercial-grade flubendiamide purchased locally was used as the test pesticide.

B. Determination of LC_0 and LC_{50} Concentrations

A 96-hour acute toxicity bioassay was conducted to determine the LC_0 and LC_{50} concentrations of flubendiamide for *Labeo rohita* fingerlings. Healthy, uniformly sized fish (8 ± 2 cm; 9 ± 2 g) were randomly allocated into three groups and maintained under identical laboratory conditions.

- Control Group**:** Fish maintained in clean dechlorinated water without pesticide exposure.
- LC_0 Group: Fish exposed to the lowest concentration of flubendiamide that produced no mortality during 96 hours.
- LC_{50} Group: Fish exposed to the concentration of flubendiamide that resulted in 50% mortality within 96 hours.

Based on preliminary range-finding tests, the LC_0 and LC_{50} values were experimentally determined to be 7 mg L^{-1} and 12 mg L^{-1}, respectively. Each group consisted of ten fish kept in 20 L glass aquaria containing fresh dechlorinated water with continuous aeration. Water quality parameters were regularly monitored throughout the exposure period to ensure consistent conditions.
After 96 hours of exposure, fish from all groups were sacrificed and liver tissue was used for histological study.

C. Histological studies

Liver tissues from all experimental groups were carefully excised and immediately fixed in 10% neutral buffered formalin for preservation of cellular architecture. The fixed samples were processed for histological analysis using standard procedures, including fixation, dehydration, clearing, embedding, sectioning, and staining [11]. Following fixation, tissues were dehydrated through a graded series of ethanol (30%–100%) and subsequently cleared in xylene until complete transparency was achieved. The cleared tissues were then infiltrated and embedded in molten paraffin wax, and paraffin blocks were prepared. Thin tissue sections (4–5 µm) were cut using a rotary microtome and mounted on glass slides. The sections were stained with hematoxylin and eosin (H&E) to visualize cellular details and structural alterations. Stained slides were mounted using DPX and examined under a compound light microscope. Photomicrographs were captured, documented, and interpreted for histopathological evaluation.

III. RESULTS

Liver sections from control *Labeo rohita* displayed normal architecture characterized by well-organized hepatic cords, polygonal hepatocytes with centrally placed nuclei, and intact sinusoids; no pathological alterations were observed (Fig. 1).
In contrast, fish exposed to flubendiamide showed dose-dependent hepatic damage. In the LC_0 group (7 mg L^{-1}; 96 h), the liver exhibited mild to moderate alterations, including disorganization of hepatic cords, occasional pyknotic nuclei, small cytoplasmic vacuoles, widening of sinusoidal spaces, and mild vascular congestion (Fig. 2). These changes indicate early hepatocellular injury: pyknotic nuclei suggest nuclear condensation associated with cell stress (apoptosis or necrosis), vacuolation likely reflects cytoplasmic degeneration (lipid or glycogen accumulation or hydropic change), and sinusoidal dilation/congestion point to circulatory disturbances within the hepatic parenchyma.
In the LC_{50} group (12 mg L^{-1}; 96 h), lesions were markedly more severe and widespread. Prominent features included extensive disarray of hepatic cords, frequent pyknotic and shrunken nuclei, pronounced cytoplasmic vacuolation, enlarged and congested sinusoids, and areas of hemorrhage (Fig. 3). The combination of extensive vacuolation, nuclear pyknosis, and hemorrhagic foci indicates advanced hepatocellular degeneration and loss of tissue integrity, consistent with dose-dependent hepatotoxicity. These findings suggest that acute exposure to higher concentrations of flubendiamide overwhelms hepatic protective mechanisms (antioxidant systems and repair processes), leading to cellular necrosis, disruption of microcirculation, and

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inflammatory/hemorrhagic responses. Overall, the histological profile ranging from mild degenerative changes at 7 mg L^{-1} to severe necrotic and hemorrhagic lesions at 12 mg L^{-1}—supports a dose-related hepatotoxic effect of flubendiamide in *L. rohita.* The observed pathology aligns with known mechanisms of pesticide toxicity: oxidative stress, membrane lipid peroxidation (LPO), mitochondrial dysfunction and impaired energy metabolism, all of which can precipitate vacuolation, nuclear degeneration, and impaired hepatic blood flow.

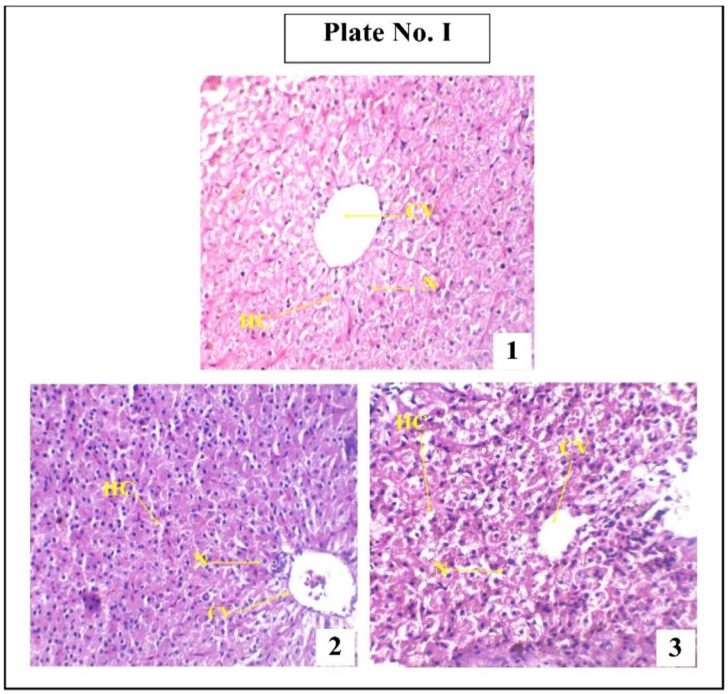
A. Plate1: Histopathological changes in Liver of control and experimental group. All the sections stained by HE.

Fig. 1: Control fish liver shows normal lobular architecture, central vein and normal arrangement of hepatic cords X 400

Fig. 2: LC_0 group (7 mg L^{-1}; 96 h), liver shows disorganization of hepatic cords, pyknotic nuclei, small cytoplasmic vacuoles X 400

Fig. 3: LC_{50} group (12 mg L^{-1}; 96 h), liver shows extensive disarray of hepatic cords, frequent pyknotic and shrunken nuclei destroyed architecture, dilation of hepatic sinusoids X 400.

Captions: CV- Central vein, N-Nucleus, HC-Hepatic cord



IV. DISCUSSION

The present investigation clearly demonstrates that acute exposure to the diamide insecticide flubendiamide results in pronounced histopathological alterations in the liver of *Labeo rohita*. Since the liver is a primary site for metabolism, detoxification, and biotransformation of xenobiotics, it is highly sensitive to toxic insults from environmental pollutants. Therefore, histopathological evaluation is widely regarded as a reliable biomarker for assessing pesticide-induced tissue damage and overall fish health.

In the current study, liver tissue from the control group exhibited normal architecture characterized by intact hepatocytes, centrally positioned nuclei, orderly hepatic cords, and clearly defined sinusoids, indicative of normal physiological function. In contrast, fish exposed to the LC_0 concentration (7 mg L^{-1}) displayed early degenerative changes including cytoplasmic vacuolation, disorganization of hepatic cords, sinusoidal dilation, vascular congestion, and pyknotic nuclei. These alterations suggest metabolic stress and compensatory responses triggered by toxic exposure. Cytoplasmic vacuolation is often associated with accumulation of lipid or glycogen due to impaired mitochondrial activity or disruption of carbohydrate metabolism during exposure to xenobiotics. At a higher exposure level (LC_{50}: 12 mg L^{-1}), more severe pathological manifestations were observed, such as extensive hepatocyte necrosis, marked vacuolation, hemorrhage, nuclear pyknosis, and distortion of hepatic parenchyma. These changes reflect irreversible cellular injury typically resulting from excessive generation of reactive oxygen species (ROS). Elevated ROS levels disrupt antioxidant defense systems, trigger lipid peroxidation of cell membranes, alter enzyme functions, and impair mitochondrial ATP synthesis, ultimately leading to programmed or necrotic cell death [12]

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Comparable findings have been reported in other fish species exposed to pesticides. Singh and Vishwakarma (2024) [13] documented hepatocyte vacuolation, necrosis, and sinusoidal expansion in *Channa punctatus* following pesticide intoxication, while Bhoi *et al.* (2021) observed severe hepatic degeneration and inflammatory responses in *Channa marulius* exposed to cypermethrin [14]. These supporting studies reinforce the current results and highlight the vulnerability of fish liver to pesticide-induced oxidative stress and metabolic disruption.

The progression of tissue damage from mild cellular degeneration at LC_0 to extensive necrosis and hemorrhage at LC_{50} suggests that increasing pesticide concentration overwhelms the liver's antioxidant and detoxification defenses. Such structural impairment may compromise essential physiological functions including metabolism, immunity, growth regulation, reproduction, and ultimately survival. Disturbances of this magnitude have the potential to adversely affect the stability of fish populations and disrupt ecological balance in contaminated aquatic ecosystems.

Overall, the findings of this study indicate that even short-term exposure to flubendiamide can exert significant hepatotoxic effects in *Labeo rohita*, underscoring potential ecological risks associated with its widespread agricultural use. These results emphasize the urgent need for controlled pesticide application, strict monitoring of agricultural runoff, and incorporation of histopathological biomarkers in aquatic toxicity assessment programs.

V. CONCLUSION

The present investigation demonstrates that acute exposure to the insecticide flubendiamide induces significant histopathological alterations in the liver of the freshwater fish *Labeo rohita*. Structural changes including cytoplasmic vacuolation, degeneration of hepatocytes, disorganization of hepatic cords, sinusoidal dilation, congested blood vessels, nuclear pyknosis, necrosis, and hemorrhagic lesions were observed, with severity increasing in a concentration-dependent manner. These pathological responses indicate impairment of liver function and disruption of cellular integrity, likely associated with oxidative stress and metabolic toxicity induced by flubendiamide. The study clearly reveals that even short-term sublethal exposure can adversely affect hepatic architecture, suggesting potential long-term ecological risks to fish health.

Considering the ecological and aquaculture importance of *Labeo rohita*, the findings highlight the necessity for careful regulation of flubendiamide usage near aquatic ecosystems and continued biomonitoring to minimize pesticide contamination. Further research focusing on chronic exposure, biochemical markers, antioxidant responses, and recovery potential is recommended to better evaluate environmental safety and toxicity thresholds.

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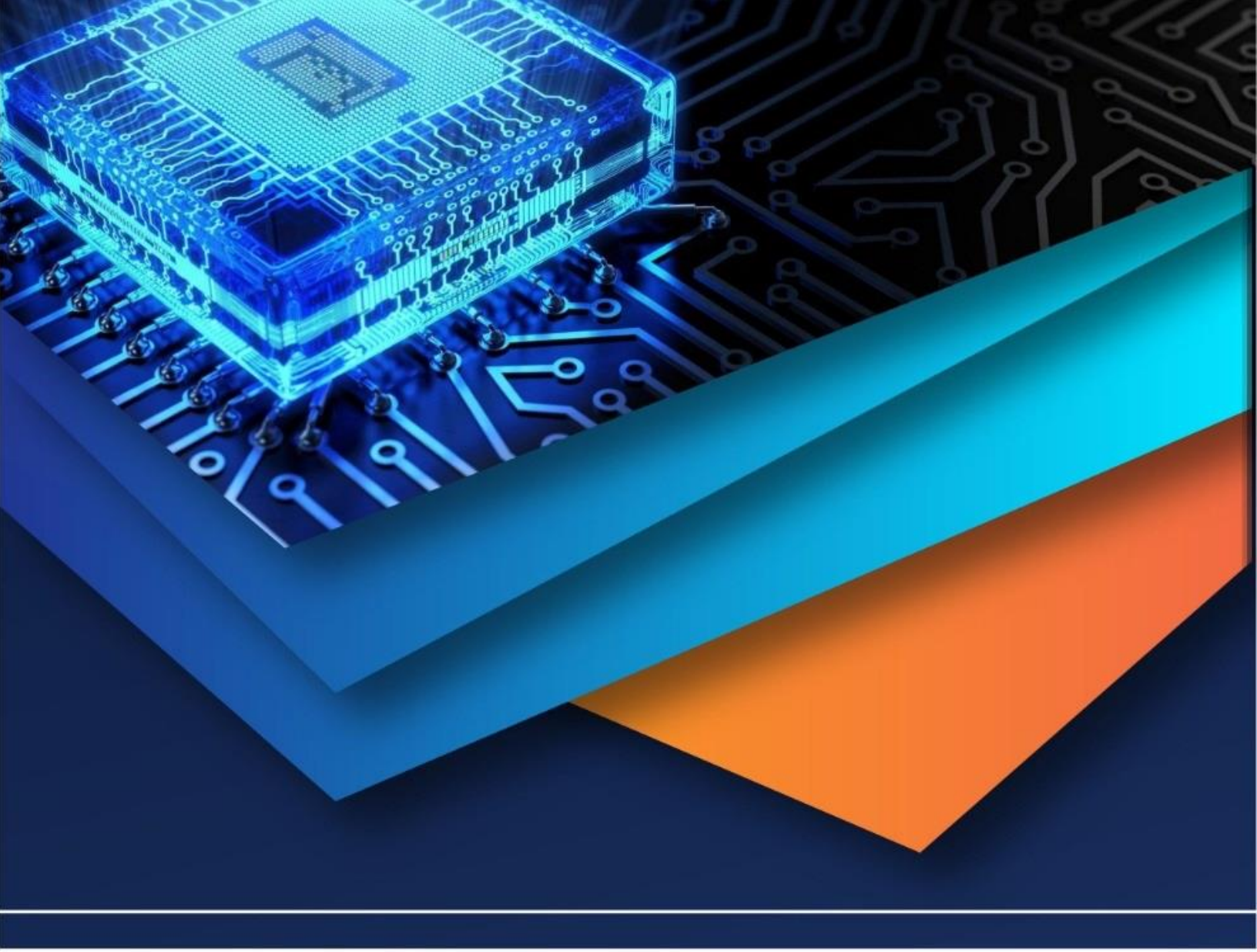

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