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TOXIC IMPACT OF FLUBENDIAMIDE ON LIPID PEROXIDATION IN THE LIVER AND BRAIN OF FRESHWATER FISH *LABEO ROHITA*

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ABSTRACT

The widespread use of the diamide insecticide flubendiamide in modern agriculture has raised concern about its potential toxicity to non-target aquatic organisms. The present study investigates the acute (96 hr) effects of flubendiamide on lipid peroxidation in the liver and brain tissues of the freshwater fish *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. Lipid peroxidation was assessed by estimating malondialdehyde (MDA) content, a key biomarker of oxidative stress. Results revealed a significant, concentration-dependent increase in MDA levels in both liver and brain tissues compared to controls, confirming the induction of oxidative stress. These findings demonstrate that even short-term exposure to flubendiamide can disrupt redox homeostasis and enhance lipid peroxidation in *L. rohita*. Hence, uncontrolled use of flubendiamide near aquatic environments may pose ecological risks to fish and other aquatic organisms. The study emphasizes the need for careful monitoring and regulation of pesticide runoff and recommends further investigations on chronic exposure and antioxidant defense mechanisms.

KEYWORDS: *Labeo rohita*, Flubendiamide, Lipid peroxidation, Malondialdehyde, Oxidative stress.

1. INTRODUCTION

Rapid agricultural intensification across the globe has led to an extensive rise in the use of pesticides to protect crops and enhance productivity. However, the excessive application of these chemicals often results in their runoff into aquatic ecosystems, where they threaten non-target organisms, including fish.[1] Once introduced into water bodies, pesticides can deteriorate water quality due to their toxicity, chemical persistence, bioaccumulative nature, and potential for biomagnification through the food chain, ultimately disturbing ecological balance.[2]

Fish are particularly vulnerable to such pollutants, as they absorb and accumulate them either directly from the surrounding water or indirectly through contaminated food.[3] The bioaccumulation of these toxicants in fish tissues can lead to a variety of physiological and biochemical disturbances, including oxidative stress, immunosuppression, endocrine disruption, and neurotoxicity, which together impair growth, reproduction, and survival.[4, 5, 6, 7]

A major mechanism underlying pesticide-induced toxicity is the generation of oxidative stress. Pesticide exposure has been shown to stimulate the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS), resulting in oxidative damage to lipids, proteins, and DNA.[8] This imbalance between pro-oxidants and antioxidants disrupts cellular redox homeostasis, overwhelms enzymatic antioxidant defenses, and initiates lipid peroxidation. Lipid peroxidation (LPO) is one of the key biochemical indicators of oxidative damage, as it involves the degradation of polyunsaturated membrane lipids and produces malondialdehyde (MDA), a reliable biomarker of oxidative stress.

Flubendiamide [N²-(1,1-dimethyl-2-(methylsulfonyl) ethyl)-3-iodo-N¹-(2-methyl-4-(1,2,2,2-tetrafluoro-1-(trifluoromethyl)ethyl)phenyl)-1,2-benzenedicarboxamide] is a modern insecticide belonging to the phthalic acid diamide group, known for its high efficacy against lepidopteran pests.[9] Its unique mode of action involves selective activation of insect ryanodine receptors (RyRs) located on neuronal and muscular membranes, triggering uncontrolled calcium ion release and paralysis in target pests.[10] This specificity also minimizes cross-resistance with other insecticide classes, making flubendiamide a popular choice in pest control programs.[11] Despite its agricultural importance, studies have shown that flubendiamide may pose potential risks to aquatic life when it enters freshwater systems. Therefore, the present study aims to evaluate the acute toxicity and oxidative stress potential of flubendiamide in the liver and brain tissues of the freshwater fish *Labeo rohita* by assessing lipid peroxidation as a biomarker of oxidative damage.

2. MATERIALS AND METHODS

2.1. Experimental Fish and Chemicals- Healthy fingerlings of the freshwater fish *Labeo rohita* were obtained from a fish seed rearing unit at Kale, Kolhapur, Maharashtra. On arrival, fish were treated with 0.1% potassium permanganate ($KMnO_4$) solution for a few minutes to eliminate external parasites and surface infections. The fish were then acclimated to laboratory conditions for 15 days in aerated glass aquaria containing dechlorinated tap water. During acclimation, they were fed daily with a commercial floating fish feed, and leftover food and fecal matter were siphoned out every day to maintain water quality. The physico-chemical parameters of the holding water such as temperature, pH, dissolved oxygen, hardness, and alkalinity were maintained within optimal limits for *L. rohita* and analyzed following the standard procedures recommended by the American Public Health Association (APHA, 1998).[12] Commercial-grade flubendiamide was procured locally and used as the test chemical.

2.2. Determination of LC_0 and LC_{50} Concentrations- An acute toxicity bioassay of 96 hours duration was performed to determine the lethal concentrations (LC_0 and LC_{50}) of flubendiamide for *Labeo rohita* fingerlings. Healthy fish with an average length of 8 ± 2 cm and weight of 9 ± 2 g were randomly distributed into three experimental groups and maintained under controlled laboratory conditions. The first group served as the Control**,** where fish were kept under identical conditions but without pesticide exposure. The second group, referred to as the LC_0 group**,** was exposed to the lowest concentration of flubendiamide that caused no mortality during the 96-hour exposure period. The third group, designated as the LC_{50} group**,** was exposed to the concentration of flubendiamide that resulted in 50% mortality within the same duration. Based on experimental observations, the LC_0 and LC_{50} concentrations were determined to be 7 mg L^{-1} and 12 mg L^{-1}, respectively. Each treatment group consisted of ten fish maintained in glass aquaria containing 20 L of dechlorinated tap water, with continuous aeration and regular monitoring of physicochemical parameters. At the end of the 96-hour exposure period, fish from each group were collected and processed for biochemical analyses.

2.3. Estimation of Lipid Peroxidation Activity- At the end of the 96-hour exposure period, fish from each experimental group (Control, LC_0, LC_{50}) were sacrificed, and their liver and brain tissues were carefully dissected and pooled separately. The extent of lipid peroxidation (LPO) was estimated according to the method described by Wills (1966).[13] This method quantifies malondialdehyde (MDA), the end product of lipid peroxidation, which reacts with thiobarbituric acid to form a pink chromogen measurable at 532 nm using a UV–visible spectrophotometer. The MDA content was expressed as nanomoles of MDA formed per milligram of tissue.

2.4. Statistical Analysis- All experimental values were expressed as mean ± standard deviation (SD). The data were statistically analyzed using one-way analysis of variance (ANOVA) to determine significant differences among treatments. A p-value < 0.05 was considered statistically significant.

3. RESULTS AND DISCUSSION

The results of the present investigation revealed a marked elevation in malondialdehyde (MDA) levels in both the liver and brain tissues of *Labeo rohita* following acute exposure (96 h) to flubendiamide. A progressive increase in MDA concentration was observed in LC_0 and LC_{50} groups compared to control group, indicating dose-dependent oxidative stress (Graph 1). In the control group**,** the MDA levels were 2.49 ± 1.10 nmol MDA/mg tissue in liver and 4.95 ± 1.19 nmol MDA/mg tissue in brain**.** In contrast, fish exposed to LC_0 (7 mg L^{-1}) exhibited MDA levels of 6.98 ± 1.21 nmol/mg in liver and 10.75 ± 1.05 nmol/mg in brain. A further increase was observed at LC_{50} (12 mg L^{-1})**,** where MDA concentrations reached 18.96 ± 1.56 nmol/mg in liver and 21.89 ± 1.23 nmol/mg in brain tissues.

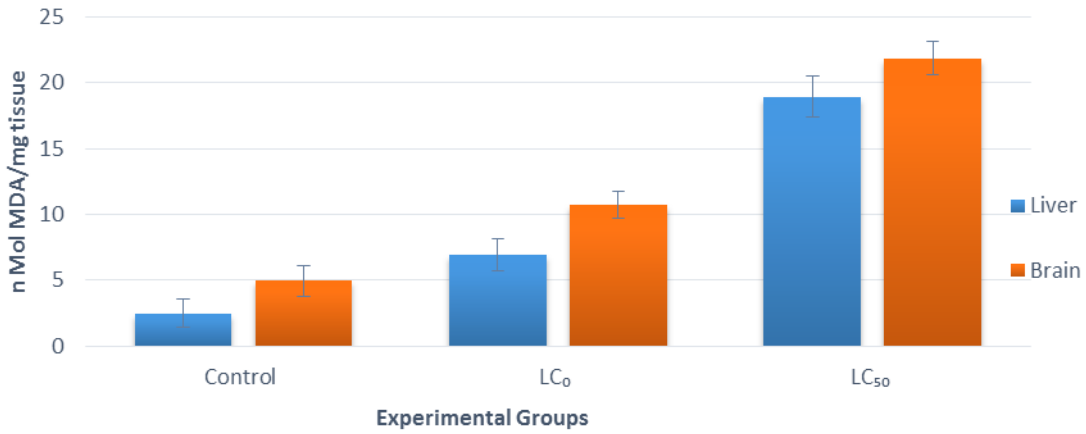
The observed elevation in MDA levels reflects enhanced lipid peroxidation (LPO), a key manifestation of oxidative stress in fish exposed to toxicants. Similar findings have been reported in various fish species exposed to pesticides, where increased MDA serves as a reliable biomarker of oxidative damage.[14-16] The liver, being the principal organ for metabolism and detoxification, is particularly susceptible to pesticide-induced oxidative injury.[17-19] Exposure to xenobiotics such as flubendiamide can alter the activity of detoxifying and antioxidant enzymes including cytochrome P450 monooxygenases, catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase

(GPx).[20,21,22,23] Disruption of these enzymatic systems leads to accumulation of reactive oxygen species (ROS) and consequent peroxidation of membrane lipids, impairing hepatic cellular function.[24]

The brain is also a critical target of oxidative stress due to its high lipid content, intense oxygen consumption, and relatively low antioxidant capacity.[25] ROS generated during pesticide exposure can oxidize neuronal membrane lipids and proteins, thereby affecting membrane fluidity, ion permeability, and neurotransmission.[26] Oxidative modification of neuronal proteins may also inhibit acetylcholinesterase (AChE), leading to abnormal accumulation of acetylcholine and disturbance of neurophysiological functions.[27-28] Moreover, mitochondrial dysfunction and reduced ATP synthesis triggered by ROS can initiate apoptotic pathways in neurons, resulting in impaired brain function and behavioral alterations.[29-30]

In the present study, the significant rise in MDA levels in both liver and brain tissues at LC_0 and LC_{50} concentrations demonstrates that even short-term exposure to flubendiamide is capable of inducing substantial oxidative stress in *Labeo rohita*. The increase was more pronounced in liver tissue, likely due to its central role in detoxification and metabolic processing of xenobiotics. These results clearly suggest that flubendiamide possesses the potential to evoke lipid peroxidation and oxidative damage in freshwater fish, thereby posing ecological risks if released into aquatic environments.

Graph. 1. Acute exposure of Flubendiamide on liver & brain tissue of *Labeo rohita*



4. CONCLUSION

The present study revealed acute exposure to the insecticide flubendiamide significantly elevates lipid peroxidation in both liver and brain tissues of *Labeo rohita*, indicating the induction of oxidative stress. The dose-dependent rise in malondialdehyde (MDA) levels observed in LC_0 and LC_{50} groups reflects disruption of cellular redox balance and enhanced formation of reactive oxygen species. As the liver is the primary organ for detoxification and metabolism, its higher susceptibility highlights the potential impact of flubendiamide on vital physiological processes. Similarly, increased oxidative stress in brain tissue suggests possible neurotoxic effects that may impair normal behavior and coordination in fish. Overall, these findings suggest that flubendiamide, though designed as a selective insecticide, can exert harmful effects on non-target aquatic organisms when introduced into freshwater systems. Proper monitoring, controlled usage, and prevention of agricultural runoff are therefore essential to minimize its ecological risks. Further studies focusing on chronic exposure, antioxidant enzyme responses, and histopathological alterations are recommended to better understand the long-term impact of flubendiamide on aquatic fauna.

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