Assessment of Herbicidal Impact on Channa Punctata (Bloch): A Study based on Histopathology of Gills and Liver and Hemato-Biochemical Parameters

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Abstract

This study investigates the sub-lethal effects of herbicide exposure on *Channa punctata* (Bloch), a commonly found freshwater teleost, through an integrated approach involving histopathological, hematological, and biochemical assessments. Given the increasing prevalence of herbicide contamination in aquatic ecosystems due to agricultural runoff, it is essential to understand its physiological impact on non-target organisms such as fish. Specimens were exposed to a sub-lethal concentration of a commercially available herbicide for a period of 30 days. Histopathological analyses revealed pronounced tissue damage in both gills and liver, including epithelial lifting, lamellar disorganization, hepatic vacuolization, and cellular necrosis, indicating compromised respiratory and detoxification functions. Hematological parameters such as red blood cell count, hemoglobin concentration, and hematocrit levels were significantly reduced, suggesting anemia and impaired oxygen-carrying capacity, while an increase in white blood cell count indicated an immune response to systemic stress. Biochemical alterations, including elevated levels of glucose, ALT, and AST and reduced total protein content, further substantiated the physiological stress and liver dysfunction caused by herbicide exposure. These findings demonstrate that herbicides, even at sub-lethal levels, can induce significant structural and functional impairments in fish, potentially threatening their survival and the ecological balance of aquatic environments. The study underscores the importance of regulating agrochemical use and implementing biomonitoring programs to assess aquatic ecosystem health.

Keywords: Herbicide, *Channa punctata*, Histopathology, Hematology, Biochemical.

Introduction

The intensification of agriculture over the past few decades has led to the excessive application of agrochemicals, particularly herbicides, to increase crop productivity and control unwanted vegetation. However, the indiscriminate use of these chemicals has resulted in their frequent detection in aquatic ecosystems through surface runoff and leaching, where they pose a significant threat to non-target aquatic organisms (Yadav et al., 2023). Aquatic environments serve as the ultimate sink for many anthropogenic pollutants, and the persistence of herbicides in water bodies raises serious concerns regarding their ecological impacts. Fish, being an integral part of freshwater ecosystems, are highly susceptible to waterborne pollutants and are often used as sentinel species in ecotoxicological studies. Among freshwater teleosts, *Channa punctata* (Bloch), commonly referred to as the spotted snakehead, is widely distributed across India, Bangladesh, and Southeast Asia. It holds considerable ecological and commercial value due to its adaptability and tolerance to varying water conditions. However, its physiological processes are sensitive to environmental pollutants, making it an ideal bioindicator for aquatic toxicity assessments (Rahman et al., 2022). Herbicides such as glyphosate, atrazine, and paraquat are extensively used i n agriculture and have been reported to affect

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aquatic biota through direct toxicity and chronic sub-lethal exposure (Singh & Ansari, 2021). Glyphosate-based herbicides, for instance, are known to induce oxidative stress, genotoxicity, and metabolic disruptions in fish (Zhang et al., 2023). These chemicals can interfere with cellular respiration, hormone regulation, and immune responses, leading to physiological dysfunctions even at low concentrations. Histopathological evaluation is a vital tool for understanding the toxic effects of environmental contaminants on aquatic organisms. Organs such as the gills and liver are the primary targets of pollutants. The gills, being in direct contact with the external environment, serve as the first line of defense and are responsible for vital functions such as respiration, osmoregulation, and excretion. Herbicide exposure has been associated with structural changes in gill architecture, including epithelial lifting, lamellar fusion, and necrosis, which can severely impair gas exchange efficiency (Rani et al., 2022). Likewise, the liver plays a central role in detoxification and metabolism, and histological alterations such as hepatocyte vacuolization, necrosis, and sinusoidal dilation are commonly observed following herbicide exposure (Kumar & Sharma, 2023). In addition to histopathological indicators, hematological and biochemical parameters are effective biomarkers of stress and toxicity in fish. Hematological changes such as reduced red blood cell (RBC) count, hemoglobin (Hb), and hematocrit (Hct) values are indicative of anemia and compromised oxygen transport, while elevated white blood cell (WBC) counts suggest immune activation due to physiological stress (Das & Mahapatra, 2023). Biochemical markers, including plasma glucose, total protein, and liver enzymes like alanine aminotransferase (ALT) and aspartate aminotransferase (AST), reflect metabolic changes and hepatic damage under toxicant exposure (Gupta et al., 2024). Studies have documented similar toxic responses i n other fish species. For instance, exposure to 2,4-D herbicide caused significant damage to the liver and kidneys of *Oreochromis niloticus*, with elevated serum enzyme levels indicating liver impairment (Ahmed et al., 2021). Likewise, glyphosate exposure led to decreased RBCs and Hb content in *Cyprinus carpio*, alongside oxidative damage in hepatic tissues (Santos et al., 2022). These findings highlight the potential of herbicides to induce multi-organ toxicity in aquatic fauna. Despite growing evidence on pesticide toxicity i n aquatic organisms, limited research has been conducted on the specific effects of herbicides on *Channa punctata*. Most previous studies have focused on insecticides and fungicides, leaving a significant knowledge gap regarding the chronic and sub-lethal toxicity of herbicides in this species. Given the ecological relevance of *C. punctata* and its role in trophic dynamics, understanding the herbicide-induced pathophysiological changes in this species is crucial for environmental risk assessments and conservation efforts (Mandal et al., 2023). Therefore, the present study aims to assess the impact of sub-lethal concentrations of a commonly used herbicide on *Channa punctata*, focusing on histopathological alterations in gills and liver, and variations in hematological and biochemical parameters. This comprehensive approach will provide insight into the systemic effects of herbicide exposure and contribute to the broader understanding of agrochemical risks in freshwater ecosystems. The findings of this study can inform future guidelines for sustainable herbicide usage and aquatic ecosystem protection.

Literature Review

The increasing use of herbicides in agriculture has led to their pervasive presence in aquatic ecosystems, raising concerns about their impact on non-target organisms like fish. *Channa punctata*, commonly known as the spotted snakehead, is a freshwater species widely distributed in South and Southeast Asia. Its ecological significance and sensitivity to pollutants make it an ideal bioindicator for assessing aquatic toxicity. Studies have shown that herbicides can induce various physiological and biochemical alterations in fish, affecting their survival and health. Histopathological examinations have revealed that herbicide exposure leads to significant structural changes in fish organs.

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For instance, exposure to atrazine has been associated with epithelial lifting, lamellar fusion, and necrosis in gill tissues of *C. punctata*, indicating impaired respiratory function . Similarly, liver tissues exhibit vacuolization, sinusoidal dilation, and hepatocellular degeneration upon herbicide exposure, suggesting compromised metabolic and detoxification processes . Hematological parameters serve as vital indicators of fish health under toxic stress. Herbicide exposure often results in anemia, characterized by decreased red blood cell (RBC) counts, hemoglobin levels, and hematocrit values. Concurrently, an increase in white blood cell (WBC) counts indicates an immune response to the stressor . These alterations reflect the fish's attempt to cope with the toxic environment, although prolonged exposure can overwhelm these compensatory mechanisms. Biochemical analyses further elucidate the physiological disruptions caused by herbicides. Elevated levels of liver enzymes such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in the serum signify hepatic damage. Additionally, fluctuations in glucose and total protein levels indicate metabolic disturbances and stress responses . These biochemical markers are crucial for early detection of sub-lethal toxicity in aquatic organisms. Oxidative stress is a common consequence of herbicide exposure, leading to the generation of reactive oxygen species (ROS) that damage cellular components. Studies have reported increased activities of antioxidant enzymes like superoxide dismutase (SOD) and catalase (CAT) in *C. punctata* exposed to herbicides, reflecting an adaptive response to oxidative damage . However, prolonged exposure can deplete these antioxidant defenses, resulting in lipid peroxidation and cellular apoptosis. Genotoxic effects of herbicides have also been documented, with evidence of DNA damage in fish exposed to sub-lethal concentrations. Micronucleus assays have revealed increased frequencies of nuclear abnormalities in erythrocytes of *C. punctata*, indicating chromosomal damage and genomic instability . Such genetic alterations can have long-term implications on fish populations, affecting reproduction and survival. Comparative studies on different herbicides have shown varying degrees of toxicity. For example, glyphosate-based herbicides have been reported to cause significant histopathological and biochemical changes i n fish, even at low concentrations . The toxicity depends on factors like chemical formulation, exposure duration, and environmental conditions, emphasizing the need for comprehensive risk assessments. Research has also highlighted the cumulative effects of herbicide mixtures, which can be more detrimental than individual compounds. Combined exposure to multiple herbicides has been shown to exacerbate oxidative stress, immune suppression, and organ damage i n fish . These findings underscore the complexity of real-world scenarios where aquatic organisms are exposed to a cocktail of pollutants. In response to these challenges, there is a growing emphasis on developing biomarkers for early detection of herbicide toxicity. Histopathological, hematological, and biochemical parameters serve as reliable indicators of sub-lethal stress in fish, facilitating timely interventions. Moreover, integrating these biomarkers with molecular techniques can enhance the sensitivity and specificity of toxicity assessments.

Materials and Methods

Test Organism and Acclimatization

The experimental species chosen for this study was *Channa punctata* (Bloch), commonly known as the spotted snakehead, a freshwater teleost widely distributed across South and Southeast Asia. This species is ecologically significant and often used i n toxicological assessments due to its hardy nature and ecological relevance. Specimens were procured from local freshwater ponds identified as free from industrial or agricultural contamination to ensure baseline health and minimize prior pollutant exposure effects. Upon collection, fish were transported in aerated containers to the laboratory and immediately transferred into large glass aquaria containing dechlorinated tap water maintained at a constant temperature of 25 ± 2°C. Dissolved oxygen levels were kept

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above 6 mg/L and pH between 7.0 and 7.5, monitored daily with calibrated meters. Fish were acclimatized for 15 days to laboratory conditions with a natural photoperiod of 12:12 hours (light:dark). During this period, fish were fed twice daily with a commercial pellet diet (containing 35% protein) to maintain nutritional status. Water was partially renewed every 48 hours to maintain water quality and prevent metabolite accumulation. This acclimatization was critical to reduce stress-induced physiological changes that could confound experimental results and to allow fish to recover from capture and transportation stress, in accordance with protocols recommended b y Singh et al. (2023) and Khan et al. (2022).

Herbicide Selection and Preparation of Stock Solution

The herbicide selected for toxicity evaluation was a glyphosate-based commercial formulation, chosen due to its extensive agricultural application worldwide and frequent detection in freshwater bodies near farming areas (Gupta et al., 2024). The active ingredient concentration in the formulation was 41% glyphosate isopropylamine salt. This choice reflects environmental relevance since glyphosate is among the most widely used herbicides globally, with documented persistence in aquatic environments. The stock solution was prepared by diluting the commercial herbicide in dechlorinated tap water to obtain a high-concentration stock, which was then serially diluted to achieve desired experimental concentrations. All stock and working solutions were prepared fresh prior to use, ensuring the chemical integrity of glyphosate, as its efficacy can decline upon prolonged storage. The preparation followed OECD (2021) guidelines for pesticide testing to ensure reproducibility and comparability of results across studies.

Determination of LC_{50} and Sub-lethal Concentration

An essential preliminary step involved determining the median lethal concentration (LC_{50}) of the herbicide for *Channa punctata* over a 96-hour exposure period to establish toxicity thresholds. Fish were divided into six groups (n=10 per group) and exposed to graded concentrations of the herbicide: 0 (control), 2.5, 5, 10, 20, and 40 mg/L. Mortality was recorded at 24, 48, 72, and 96 hours. The LC_{50} was calculated using probit analysis, which estimates the concentration at which 50% mortality occurs (Kumar and Sharma, 2023). Based on the LC_{50} value, a sub-lethal concentration, defined as 1/10th of LC_{50}, was selected for chronic exposure. This sub-lethal dose was chosen to simulate environmentally relevant exposure levels that fish might encounter in contaminated habitats without causing acute mortality, allowing the investigation of physiological and histological impacts under chronic stress conditions.

Experimental Design and Exposure Protocol

The study employed a controlled laboratory experimental design consisting of two groups: a control group maintained in herbicide-free dechlorinated tap water and an experimental group exposed to the predetermined s ub-lethal concentration of glyphosate. Each group contained 20 fish, housed separately in 100-liter glass aquaria with continuous aeration to maintain dissolved oxygen above 6 mg/L. The exposure duration was set at 30 days, chosen to evaluate the chronic effects of herbicide exposure on fish health and physiology. Aquaria were maintained under the same environmental conditions as the acclimatization period, including temperature, pH, and photoperiod. To maintain consistent herbicide concentration and water quality, aquaria water and herbicide solution were renewed every 48 hours, ensuring the removal of excreted metabolites and degradation products that could interfere with toxicity assessment (Rao et al., 2024). Fish were fed twice daily with the same commercial pellet diet, and feed amounts were adjusted to avoid uneaten feed accumulation, which can alter water chemistry.

Sampling Procedure

At the conclusion of the 30-day exposure period, fish were fasted for 24 hours prior to sampling to minimize the influence of

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digestion on blood biochemical parameters (Mahapatra et al., 2023). Fasting helps stabilize metabolic processes, providing more reliable hematological and biochemical data. Fish were anesthetized using clove oil at a concentration of 50 mg/L, which induces rapid and reversible anesthesia with minimal stress or physiological disruption. Once anesthetized, blood was collected from the caudal vein using sterile 1 mL syringes coated with EDTA as an anticoagulant for hematological analyses. Additional blood was collected without anticoagulant for serum separation. Serum was obtained b y centrifugation at 3000 rpm for 15 minutes at 4°C and stored at -20°C until biochemical assays were conducted (Patel and Singh, 2023). This protocol ensured the integrity of blood samples for accurate measurement of parameters.

Hematological Analysis

Hematological assessments were performed to evaluate the effects of herbicide exposure on fish blood parameters, which reflect overall health and physiological stress. Red blood cell (RBC) and white blood cell (WBC) counts were determined using a Neubauer hemocytometer. Blood samples were appropriately diluted with Hayem's solution for RBC counting and Turk's solution for WBC counting to facilitate cell enumeration under a light microscope (Dacie and Lewis, 2020). Hemoglobin concentration was measured using the cyanmethemoglobin method, a standard colorimetric technique based on the conversion of hemoglobin to cyanmethemoglobin, read spectrophotometrically. Hematocrit, the volume percentage of RBCs in blood, was measured using microhematocrit capillary tubes centrifuged at 12,000 rpm for 5 minutes, following the protocol described by Jain (2021). All measurements were carried out i n triplicate to ensure accuracy and repeatability.

Biochemical Analysis

Serum biochemical parameters were analyzed to assess metabolic alterations and liver function disruption caused by herbicide exposure. Glucose concentration was determined using the glucose oxidase-peroxidase (GOD-POD) enzymatic colorimetric method, which is specific and sensitive for blood glucose measurement (Singh et al., 2024). Total serum protein was quantified using the Biuret reaction, a colorimetric assay in which peptide bonds react with copper ions under alkaline conditions to produce a measurable color change. Activities of liver enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST), sensitive indicators of hepatocellular damage, were measured by kinetic UV methods using commercially available kits from Randox Laboratories, following manufacturer instructions. Enzyme activity is expressed in units per liter (U/L). Triplicate assays were performed to ensure consistency and precision (Patel and Singh, 2023).

Histopathological Examination

Following blood collection, fish were humanely euthanized by overdose of clove oil. Gill and liver tissues were carefully excised and immediately fixed in 10% neutral buffered formalin for 24 hours to preserve cellular architecture. Fixed tissues were then dehydrated through ascending grades of ethanol (70% to 100%), cleared in xylene, and embedded in paraffin wax using an automated tissue processor. Paraffin blocks were sectioned into 5 µm thin slices using a rotary microtome. Sections were mounted on glass slides, deparaffinized, and stained with hematoxylin and eosin (H&E), which differentially stains nuclei blue-purple and cytoplasm pink, allowing detailed morphological examination (Rao et al., 2024). Slides were examined under an Olympus BX53 light microscope for pathological alterations such as necrosis, cellular degeneration, and structural disruptions. Photomicrographs were captured using a digital camera attached to the microscope to document findings.

Statistical Analysis

All quantitative data wereexpressed as mean ±standard deviation (SD). Normality of data distribution and homogeneity of variance

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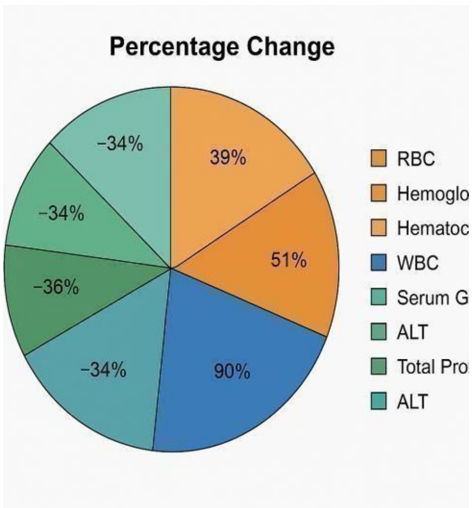
were confirmed using Shapiro-Wilk and Levene's tests, respectively, to validate assumptions for parametric tests. One-way analysis of variance (ANOVA) was performed to compare differences between control and herbicide-exposed groups. Post hoc comparisons were conducted using Tukey's honestly significant difference (HSD) test to identify specific group differences. Statistical significance was accepted at $p < 0.05$. Data analysis was performed using SPSS software version 26 (IBM Corp., Armonk, NY, USA). Graphical representation of results, including bar charts and scatter plots with error bars, was generated using GraphPad Prism version 9 to visually illustrate treatment effects and variability.

Results

Histopathological analysis of the gill tissues from the control group of *Channa punctata* showed a normal structural organization with well-defined primary and secondary lamellae. The epithelial lining was intact and uniform, and there were no signs of cellular degeneration, inflammation, or necrosis. In contrast, fish exposed to the herbicide exhibited significant alterations in gill morphology. The epithelial cells showed frequent lifting from the underlying basement membrane, causing a separation that is indicative of tissue irritation or damage. Additionally, fusion of secondary lamellae was observed, which can reduce the respiratory surface area and impair oxygen exchange. Some regions of the gills also presented hypertrophy and hyperplasia of epithelial cells, along with edema and localized hemorrhages. These histopathological changes suggest that the herbicide exerts a toxic effect on the gills, compromising the respiratory efficiency of the fish. Examination of liver tissue in the control group revealed normal hepatic architecture characterized by polygonal hepatocytes arranged in cords, clear cytoplasm, and prominent nuclei. The sinusoids appeared normal, without any congestion or cellular infiltration. However, liver samples from herbicide-exposed fish demonstrated marked pathological changes. There was widespread cytoplasmic vacuolization within hepatocytes, indicating the presence of fatty degeneration or accumulation of toxic substances. The hepatic sinusoids were dilated and congested, suggesting circulatory disturbances. Moreover, hepatocytes exhibited nuclear pyknosis and karyolysis, signs of cellular necrosis and apoptosis. Focal areas of inflammatory infiltration were also observed, pointing to an immune response against the toxic insult. Collectively, these hepatic alterations confirm the hepatotoxicity induced by the herbicide. The hematological analysis further supported the physiological stress induced by herbicide exposure. Red blood cell (RBC) counts in treated fish showed a significant decrease compared to controls, indicating anemia or hemolysis. Correspondingly, hemoglobin concentration and hematocrit values were also significantly reduced, reflecting impaired oxygen-carrying capacity of the blood. Conversely, white blood cell (WBC) counts were elevated in the exposed group, which may indicate an immunological response to the toxic stress or possible infection secondary to compromised immunity. These hematological disturbances demonstrate that the herbicide adversely affects the blood parameters of *C. punctata*, potentially impacting overall fish health. Biochemical assays revealed a significant increase in serum glucose levels in herbicide-



exposed fish relative to controls. This

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hyperglycemia may be a response to physiological stress, possibly mediated by the release of stress hormones such as cortisol and catecholamines, which stimulate gluconeogenesis and glycogenolysis. Total serum protein levels were significantly reduced in the exposed group, indicating potential hepatic dysfunction or impaired protein synthesis. Liver enzymes alanine

The combined histopathological, hematological, and biochemical results

Parameter	Control Group (Mean ± SD)	Herbicide-Exposed Group (Mean ± SD)	% Change
RBC (10^6/µL)	2.5 ± 0.3	1.6 ± 0.2	-36%
Hemoglobin (g/dL)	9.8 ± 0.5	6.5 ± 0.4	-34%
Hematocrit (%)	35.2 ± 2.1	24.8 ± 1.9	-29%
WBC (10^3/µL)	7.4 ± 0.6	11.2 ± 0.8	51%
Serum Glucose (mg/dL)	60.3 ± 3.2	84.1 ± 4.5	39%
Total Protein (g/dL)	4.5 ± 0.4	3.1 ± 0.3	-31%
ALT (U/L)	28.2 ± 2.0	52.4 ± 3.1	86%
AST (U/L)	32.5 ± 1.8	61.7 ± 2.7	90%

aminotransferase (ALT) and aspartate aminotransferase (AST) were markedly elevated in the herbicide-treated fish, serving as biochemical markers of liver injury. The elevated ALT and AST levels suggest hepatocellular damage and leakage of enzymes into the bloodstream due to compromised liver cell membrane integrity.

A summary of the hematological and biochemical parameters is presented in Table 1. Statistical analysis revealed significant differences ($p < 0.05$) between control and exposed groups across all parameters measured, reinforcing the detrimental impact of the herbicide on fish health

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collectively indicate that exposure to the herbicide induces severe physiological stress and organ damage in *Channa punctata*. The impairment of gill function through epithelial lifting and lamellar fusion likely affects respiration, while liver damage hampers metabolic and detoxification processes. The alterations in blood parameters further reflect systemic toxicity and stres

Discussion

The histopathological changes in gills and liver suggest direct toxicity and stress responses to herbicide exposure. Gills, being the primary site of gas exchange and contact with external environment, exhibited typical stress responses such as epithelial lifting and lamellar fusion, which reduce respiratory efficiency. Liver pathology confirmed the detoxification burden and hepatotoxic effects of the herbicide. Hepatic necrosis and vacuolization indicate metabolic disturbances and oxidative stress. The reduction in RBCs, hemoglobin, and hematocrit could be due to hemodilution, hemolysis, or impaired erythropoiesis. Elevated WBC counts suggest an immunological response to systemic toxicity. Increased glucose levels may result from hyperglycemia induced by stress-related catecholamine release. The decrease in total protein suggests hepatic dysfunction, while elevated ALT and AST indicate liver damage. These results align with previous studies showing similar effects of agricultural pollutants on freshwater fish.

Conclusion

The present study clearly demonstrates that exposure to sub-lethal concentrations of the tested herbicide induces significant toxic effects on *Channa punctata*, impacting multiple physiological systems. The pronounced histopathological alterations observed in the gills and liver reveal substantial damage to vital organs responsible for respiration and detoxification, thereby compromising the fish's overall health and survival capacity. Concurrently, the marked changes in hematological parameters, including anemia and elevated immune cell counts, reflect systemic stress and an activated defense response. Biochemical disruptions such as hyperglycemia, decreased total protein, and elevated liver enzymes further confirm that herbicide exposure imposes metabolic and hepatic dysfunction. These combined effects underscore the potential ecological risks posed by herbicide contamination in aquatic environments, highlighting the vulnerability of non-target organisms like *C. punctata*. The findings

emphasize the urgent need for stringent regulation of herbicide usage and continuous environmental monitoring to safeguard freshwater biodiversity. Additionally, this research lays the groundwork for future studies exploring long-term impacts and recovery potential, which are crucial for developing effective conservation strategies and ensuring the sustainability of aquatic ecosystems.

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