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Sublethal Carbofuran Effects on Liver and Blood Parameters of *Clarias batrachus*

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Abstract

This study looked into how sublethal exposure to carbofuran affects the biochemistry, blood health, and tissue structure of the freshwater fish *Clarias batrachus*. The fish were exposed to carbofuran for varying periods of 7, 15, 30, and 60 days, and we measured changes in their serum biochemical markers, blood parameters, and liver tissue structure. We found a significant increase in liver enzymes (AST and ALT) and a steady drop in total protein levels in the serum, which points to liver issues. The blood analysis showed lower hemoglobin levels and a decrease in red blood cell count, indicating anemia. When we examined the liver tissue under a microscope, we observed signs of damage, including vacuolization of liver cells, dilation of blood vessels, cell degeneration, and necrosis in the treated fish. These results clearly show that carbofuran can harm the liver and emphasize the importance of using integrated biomarkers to keep an eye on pesticide pollution in freshwater environments.

Keywords: Carbofuran; *Clarias batrachus*; Hepatotoxicity; Biochemical biomarkers; Histopathology

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Introduction

In modern farming, the heavy reliance on pesticides has greatly heightened the risk of chemical contamination in aquatic ecosystems. Carbamate pesticides, in particular, are commonly used because they work well against a wide array of insect pests. Carbofuran, a highly toxic carbamate insecticide, can enter freshwater bodies through agricultural runoff, leaching, and improper disposal methods. Once it's in the water, carbofuran can pose significant dangers to non-target organisms, especially fish, by interfering with crucial physiological and metabolic processes. Fish are considered some of the best indicators of pollution in our waters because they're highly sensitive to environmental toxins and can accumulate harmful substances in their bodies. When exposed to pesticides, fish can experience oxidative stress, enzyme dysfunction, blood problems, and tissue damage, which can all negatively impact their growth, reproduction, and survival rates. That's why it's vital to conduct toxicological assessments on fish exposed to pesticides to gauge the health of freshwater ecosystems. *Clarias batrachus*, or the air-breathing catfish, is widely found in freshwater environments throughout India and is known for its significant commercial and nutritional value. This species often resides in water bodies contaminated with pesticides, particularly in regions where intensive agriculture is practiced. Due to its resilience and ecological importance, C. batrachus is frequently used as a model organism in ecotoxicology studies. However, extended exposure to pesticides such as carbofuran can push its adaptive limits, resulting in severe physiological stress and damage to its organs. The liver plays a crucial role in detoxifying substances and metabolizing foreign compounds, making it particularly vulnerable to damage from pesticides. To evaluate liver dysfunction in fish, we often look at changes in various biochemical markers, such as serum transaminases (AST and ALT), phosphatases (ALP and ACP), lactate dehydrogenase (LDH), and protein levels. Additionally, shifts in blood parameters like hemoglobin levels and red blood cell counts can reveal important information about the fish's overall health and stress responses. A closer look at liver tissue through histopathological examination provides concrete evidence of cellular and structural damage, which helps to clarify the biochemical and hematological results. This study is designed to assess the biochemical, hematological, and histopathological effects of sublethal exposure to carbofuran in *Clarias batrachus*. A thorough evaluation of these biomarkers will enhance our understanding of the toxicity linked to carbofuran and underscore its possible ecological dangers in freshwater ecosystems.

Materials and Methods

Chemicals- Carbofuran (analytical grade, ≥99% purity) was procured from an authorized supplier. All other chemicals and reagents used for biochemical and hematological estimations were of analytical grade and obtained from standard commercial sources. Double-distilled water was used for the preparation of all solutions.

Experimental Fish-Adult specimens of the freshwater air-breathing catfish, *Clarias batrachus* were collected from local freshwater bodies. To ensure consistency, we picked fish that were all about the same size, measuring between 22 and 25 cm long and weighing between 70 and 110 grams.

Acclimatization and Maintenance- Fish were acclimatized for 15 days in glass aquaria containing dechlorinated tap water under laboratory conditions. During acclimatization, fish were fed a commercial pellet diet once daily. Water was renewed on alternate days, and feeding was discontinued 24 h prior to the experiment. Only healthy and active fish were used for experimentation.

Determination of Median Lethal Concentration (LC_{50})-The 96-h median lethal concentration (LC_{50}) of carbofuran for C. batrachus was determined using a standard static bioassay method. Fish were exposed to different concentrations of carbofuran, and mortality was recorded at 24, 48, 72, and 96 h. The LC_{50} value was calculated by probit analysis.

Experimental Design- Based on the 96-h LC_{50} value, a sublethal concentration corresponding to one-tenth (1/10) of the LC_{50} was selected. Fish were randomly divided into the following groups:

- Control group (no carbofuran exposure)
- Acute exposure group (24 h)
- Sub-acute exposure groups (7, 15, and 30 days)
- Sub-chronic exposure groups (60 days)

All groups were maintained under identical laboratory conditions through out the experimental period.

Blood Collection and Serum Separation- At the end of each exposure period, the fish were put under anesthesia, and we collected blood samples by puncturing the caudal vein with sterile syringes. For hematological analysis, we gathered blood in EDTA-coated vials, while the rest was left to clot at room temperature for serum separation. We obtained the serum by centrifuging it at 2000 rpm for 15 minutes and stored it at 4 °C until we were ready for biochemical analysis.

Hematological Analysis- Hematological parameters were analyzed using standard laboratory methods. The parameters evaluated included hemoglobin concentration (Hb), total erythrocyte count (RBC), total leukocyte count (WBC), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC).

Biochemical Analysis- Serum biochemical parameters were estimated using standard colorimetric methods and commercially available diagnostic kits.

Liver Enzyme Assays-To evaluate liver function and cellular integrity, we looked at the levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), acid phosphatase (ACP), and lactate dehydrogenase (LDH).

Protein, Bilirubin, and Lipid Profile-Total protein, albumin, globulin, albumin/globulin (A/G) ratio, total bilirubin, cholesterol, and triglycerides were analyzed to assess metabolic and functional alterations induced by carbofuran exposure.

Tissue Collection-Following blood sampling, fish were dissected, and liver tissues were excised carefully. The tissues were washed in physiological saline to remove adhering blood and immediately processed for histopathological examination.

Histopathological Examination-Liver tissues were fixed in Bouin's fixative for 24 h, dehydrated through a graded ethanol series, cleared in xylene, and embedded in paraffin wax. Sections of 5 µm thickness were prepared using a rotary microtome and stained with Hematoxylin and Eosin (H&E). The stained sections were examined under a light microscope, and representative photomicrographs were documented for comparative analysis.

Ethical Statement-All the experimental procedures were carried out following both institutional and national ethical guidelines for the care and use of experimental animals. We took all necessary steps to reduce stress and ensure that the fish were handled humanely throughout the study.

Results

Biochemical Alterations-Sublethal exposure to carbofuran led to marked changes in the serum biochemical profile of *Clarias batrachus* when set against the control group. The activities of key liver enzymes, such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT), showed a significant and ongoing rise with longer exposure periods. This uptick in enzyme levels points to liver cell damage and increased membrane permeability in the fish treated with carbofuran. serum total protein levels declined progressively with prolonged exposure. The reduction in protein content suggests impaired protein synthesis and metabolic dysfunction associated with carbofuran-induced liver damage. The changes were minimal during short-term exposure but became more pronounced during longer exposure periods.

Table 1. Serum Biochemical Parameters in Control and Carbofuran-Treated *Clarias batrachus*

Parameter	Control	7 Days	15 Days	30 Days	60 Days
AST (IU/L)	42.3 ± 2.1	58.6 ± 3.4	69.2 ± 4.1	81.5 ± 5.2	96.4 ± 6.0
ALT (IU/L)	35.1 ± 1.9	49.8 ± 2.7	61.3 ± 3.5	73.6 ± 4.6	88.2 ± 5.4
Total Protein (g/dl)	6.8 ± 0.3	5.9 ± 0.4	5.1 ± 0.3	4.4 ± 0.2	3.7 ± 0.2

Values are expressed as mean ± SD.

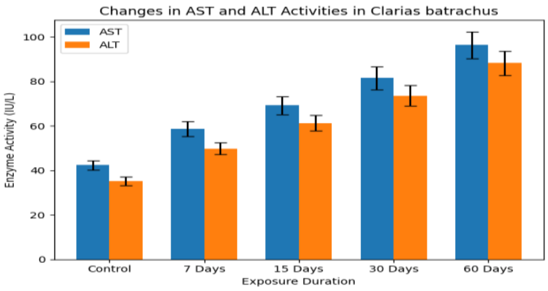

Figure 1-Changes in AST and ALT activities in *Clarias batrachus* following carbofuran exposure.Bar graph showing a progressive increase in enzyme activity with exposure duration.

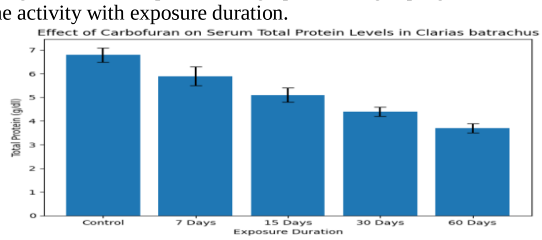

Figure 2 Effect of carbofuran exposure on serum total protein levels in *Clarias batrachus*.Bar graph showing a gradual decline in protein concentration with increasing exposure duration.

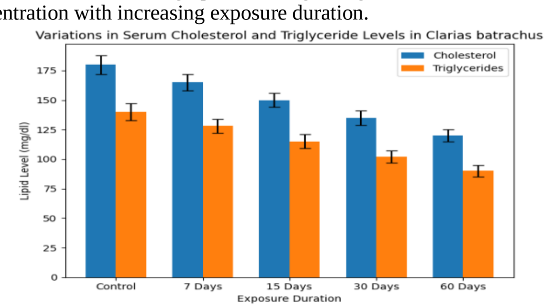

Figure 3 Variations in serum cholesterol and triglyceride levels after carbofuran exposure.Bar graph illustrating disruption of lipid metabolism.

Hematological Changes-Carbofuran exposure led to significant changes in the blood of *Clarias batrachus*. We noticed a marked drop in hemoglobin levels and a decrease in red blood cell (RBC) count in the groups that were treated, especially when compared to the control group. These findings suggest that the fish are developing anemia and their blood's ability to carry oxygen is compromised due to the stress caused by the pesticide. Moreover, the extent of these blood changes worsened the longer the exposure lasted, indicating that carbofuran has cumulative toxic effects.

Histopathological Observations (Liver)-Histological examination of liver tissue from control fish showed normal hepatic architecture characterized by well-organized hepatocytes, intact hepatic cords, and normal sinusoids. In contrast, liver sections from carbofuran-exposed fish exhibited progressive pathological alterations depending on exposure duration. The major histopathological changes observed included:

- •Hepatocyte vacuolization
- •Sinusoidal dilation
- •Cellular degeneration
- •Hepatocellular necrosis
- •Disruption of hepatic cords

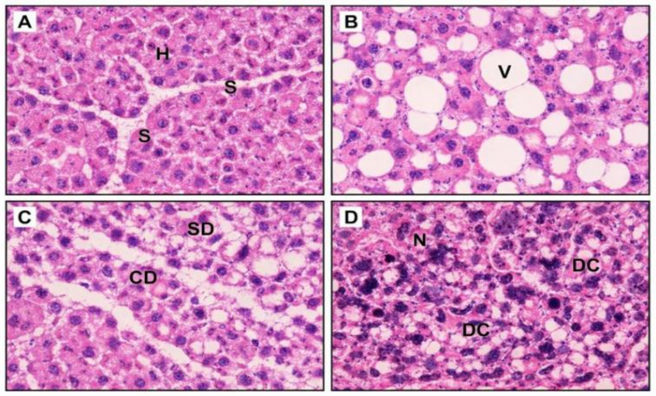

Figure 4-Histopathological alterations in liver tissue of *Clarias batrachus* exposed to carbofuran (H&E).

- •A: Control—normal hepatocytes and sinusoids
- •B: 15 days—hepatocyte vacuolization
- •C: 30 days—sinusoidal dilation and cellular degeneration
- •D: 60 days—necrosis and disrupted hepatic cords

(Magnification ×400)

Discussion

This study shows that even sublethal doses of carbofuran can cause significant changes in the biochemical, hematological, and tissue structures of *Clarias batrachus*, confirming its harmful effects on the liver and the body as a whole. The changes noted were dependent on how long the exposure lasted, indicating that longer exposure leads to greater toxic stress. The marked increase in serum AST and ALT activities in fish treated with carbofuran suggests that their liver cells are suffering damage and that the membranes are becoming more permeable. Normally, these enzymes are contained within the liver cells, so when they show up in higher amounts in the bloodstream, it signals liver dysfunction. Similar spikes in aminotransferase levels have been documented in fish exposed to carbamate and organophosphate pesticides, indicating that carbofuran disrupts the liver's metabolic and detoxifying functions (Banaee *et al*., 2011; Jiraungkoorskul *et al*., 2003). The gradual rise in enzyme activities with prolonged exposure observed in this study further supports the idea of ongoing liver injury. We also observed a significant drop in serum total protein levels, which suggests that protein synthesis is impaired and there's some metabolic dysfunction going on. Since the liver is the main hub for protein production, lower protein levels point to some issues with liver function. In fish exposed to pesticides, the reduced protein content has been linked to decreased transcriptional activity and an uptick in protein breakdown due to toxic stress (Saravanan *et al*., 2011). Hematological issues, especially drops in hemoglobin levels and red blood cell counts, indicate that fish exposed to carbofuran may be developing anemia. These changes could stem from either hindered red blood cell production or increased destruction of these cells, which ultimately leads to less efficient oxygen transport and metabolism. Blood parameters are well-known as sensitive indicators of pesticide-related stress in aquatic life (Adhikari *et al*., 2004). Changes in lipid profiles, shown by shifts in serum cholesterol and triglyceride levels, point to a disruption in lipid metabolism due to carbofuran exposure. Lipids are crucial for energy metabolism, and any imbalance can indicate metabolic stress and oxidative damage (Atamanalp *et al*., 2011). The histopathological analysis of liver tissue revealed clear signs of toxicity caused by carbofuran. The treated fish showed hepatocyte vacuolization, dilation of the sinusoids, cellular degeneration, and necrosis, which align with the biochemical results and confirm that the liver is a key target organ for carbofuran toxicity.

Conclusion

The current research highlights that even low levels of carbofuran exposure lead to significant biochemical, hematological, and histopathological issues in the freshwater fish *Clarias batrachus*. This finding underscores the toxic effects of this commonly used carbamate pesticide. The changes observed were linked to the duration of exposure, suggesting that prolonged contact with the pesticide results in cumulative physiological stress. A significant rise in liver enzymes like AST and ALT pointed to liver function issues and increased membrane permeability, which indicates damage to liver cells. The drop in serum total protein levels also suggested that protein synthesis and metabolic processes were disrupted, highlighting the liver as a key target for carbofuran toxicity. Changes in blood parameters, such as lower hemoglobin levels and a decrease in red blood cell count, hinted at the development of anemia and a reduced ability to transport oxygen. This could negatively impact the growth, metabolism, and overall survival of fish. Alterations in the serum lipid profile, marked by variations in cholesterol and triglyceride levels, suggested that there were disruptions in lipid metabolism and energy regulation due to pesticide stress. Such metabolic disturbances can negatively affect the physiological performance and adaptability of aquatic organisms. A histopathological analysis of liver tissue confirmed the toxicity from carbofuran, showing progressive structural damage like hepatocyte vacuolization, sinusoidal dilation, cellular degeneration, and necrosis in the affected fish. These morphological changes supported the biochemical and hematological findings, enhancing the reliability of the integrated biomarker approach. Assessing biochemical, hematological, and histopathological parameters together has shown to be an effective way to evaluate the toxicity of pesticides in freshwater fish. The results reveal the ecological threats that carbofuran contamination poses to aquatic environments and highlight the importance of enforcing stricter pesticide regulations. Ongoing monitoring of freshwater ecosystems is vital to protect fish health, maintain aquatic biodiversity, and promote environmental sustainability.

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References

Adhikari, S., Sarkar, B., Chatterjee, A., Mahapatra, C. T., & Ayyappan, S. (2004). Effects of cypermethrin and carbofuran on certain hematological parameters of *Labeo rohita*. Ecotoxicology and Environmental Safety, 58(2), 220–226.

Atamanalp, M., Köse, S., & Türkmen, M. (2011). Alterations in fish biochemical parameters under pesticide stress. Pesticide Biochemistry and Physiology, 99(3), 231–236. https://doi.org/10.1016/j.pestbp.2010.12.006

Banaee, M., Mirvagefei, A. R., Rafei, G. R., & Majazi Amiri, B. (2011). Effect of sub-lethal diazinon concentrations on blood plasma biochemistry. Comparative Biochemistry and Physiology Part C, 153(1), 40–45.

Begum, G. (2004). Carbofuran insecticide induced biochemical alterations in liver and muscle tissues of the fish *Clarias batrachus* (Linn.). Pesticide Biochemistry and Physiology, 78(1), 17–26. https://doi.org/10.1016/j.pestbp.2003.09.002

Dutta, H. M., & Dalal, R. (2008). The effect of endosulfan on fish organs: A histopathological perspective. Environmental Pollution, 155(2), 221–227. https://doi.org/10.1016/j.envpol.2007.10.017

Jiraungkoorskul, W., Upatham, E. S., Kruatrachue, M., Sahaphong, S., Vichasri-Grams, S., & Pokethitiyook, P. (2003). Biochemical and histopathological effects of glyphosate herbicide on Nile tilapia (*Oreochromis niloticus*). Environmental Toxicology, 18(4), 260–267. https://doi.org/10.1002/tox.10123

Saravanan, M., Kumar, K. P., & Ramesh, M. (2011). Haematological and biochemical responses of fish exposed to pesticides. International Journal of Integrative Biology, 10(2), 80–83.

Singh, N. N., Srivastava, A. K., & Kumar, A. (2011). Toxic effects of carbofuran on hematological and biochemical parameters of freshwater fish. Journal of Environmental Biology, 32(3), 353–356.

Singh, B., Maheshwari, M., & Sharma, H. (2024). Nutritional studies on fish Catla catla found in Ganga River with hydrobiological studies. Journal of Science Innovations and Nature of Earth, 4, 17–22. https://doi.org/10.59436/ht5mmg32

Singh, B., Sharma, H. N., Prveen, & Singh, S. (2024). A review on effect of cypermethrin on hematology of fresh water fish Channa punctatus (Bloch.). Journal of Science Innovations and Nature of Earth, 4. https://doi.org/10.59436/jsiane.271.2583-2093

Tripathi, G., & Verma, P. (2004). Carbofuran-induced changes in serum biochemical composition of the freshwater fish *Clarias batrachus*. Journal of Ecophysiology and Occupational Health, 4, 47–52.