J. Sci. Innov. Nat. Earth: Vol. 5, Issue 1, 2025, Page 86-88 ISSN (Online) 2583-2093 OPEN ACCESS

Journal of Science Innovations and Nature of Earth

Journal homepage : https://jsiane.com/index.php/files/index

International, Double-Blind, Quarterly, Peer-Reviewed, Refereed Journal, Edited and Open Access Research Journal

Assessment of Carbofuran-Induced Changes in Hepatic Lipid Parameters of *Clarias batrachus* over Progressive Exposure Durations

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DOI: https://doi.org/10.59436/jsiane.d2o.2583-2093

Abstract

Aquatic ecosystems are facing increasing threats from pesticide pollution, especially from carbofuran a carbamate pesticide known for its high solubility and persistence, commonly used in farming. In this study, we looked at how carbofuran impacts the liver function of *Clarias batrachus*, a freshwater fish often used in toxicity studies. We exposed these fish to various sublethal doses of carbofuran over different time periods (1, 7, 15, 30, 45, and 60 days) and measured changes in their lipid profiles, specifically cholesterol, triglycerides, high-density lipoprotein (HDL), and low-density lipoprotein (LDL). The results showed significant increases ($p < 0.05$) in total cholesterol, triglycerides, and LDL levels, while HDL levels consistently dropped. This suggests that lipid metabolism was disrupted, leading to oxidative stress and liver problems. These changes can be linked to the reactive oxygen species produced when carbofuran is metabolized. Our findings indicate that *Clarias batrachus* is a sensitive indicator of aquatic pollution. This study highlights the urgent need for tighter environmental monitoring and regulations regarding pesticide usage to safeguard aquatic health.

Keywords*:* Carbofuran, Hepatic, Lipid Parameters, *Clarias batrachus*, Durations

Received 16.01.2025 **Revised 18.02.2025** **Accepted 05.03.2025**

Introduction

Aquatic ecosystems are facing greater threats than ever before, largely due to the careless application of agricultural pesticides. These chemicals often make their way into freshwater bodies via surface runoff and leaching. One notable example is carbofuran, a carbamate pesticide commonly used to control certain insects and nematodes across a wide range of crops because of its effectiveness. Yet, its ability to dissolve easily in water and linger in the environment raises serious red flags about its potential dangers to non-target aquatic life, especially fish (Arjmandi *et al*., 2010). Fish are like the canaries in the coal mine when it comes to spotting pollution in our waters, and they're often the go-to choice for evaluating how pesticides affect ecosystems. Take the freshwater catfish, *Clarias batrachus*, for example—this bottom-dwelling omnivore is key in the aquatic food web. Given its ecological significance, broad range, and ability to thrive in diverse conditions, it frequently pops up in toxicology studies (Javed & Usmani, 2015). Just like in other vertebrates, the liver of fish is essential for metabolism and detoxifying harmful substances. It's the main organ that transforms and eliminates foreign compounds. Because of this, hepatocytes are the first to show changes in structure and function when faced with toxic stress (Pandey *et al*., 2003). When exposed to pesticides like carbofuran, our liver can face some serious issues, showing up as changes in various biochemical markers and enzyme activities. Key enzymes like alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) are used to check liver health, and when their levels spike in the blood, it usually means there's damage to liver cells (Reddy & Yellamma, 1991). Moreover, during the breakdown of pesticides, reactive oxygen species (ROS) are produced, which can lead to oxidative stress in liver tissues. To combat this, our body activates an antioxidant system made up of superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH) to help neutralize the ROS. However, if someone is exposed to carbofuran in high doses or for a long time, these protective systems can get overwhelmed, resulting in lipid peroxidation (LPO) and cellular damage (Gül *et al*., 2004). Several studies have shown that even low levels of pesticides can lead to serious biochemical and histopathological changes in the livers of fish. This not only hampers their physiological functions but also makes them more susceptible to secondary infections and increased mortality rates (Tilak *et al*., 2001; Kaur & Sandhu, 2014). However, there's still a gap in research when it comes to understanding how carbofuran affects hepatic stress biomarkers in *Clarias batrachus* over different exposure durations. The current study is focused on examining how exposure to carbofuran affects liver function in *Clarias batrachus* by looking at important biochemical stress markers. This research not only sheds light on the hepatotoxic effects of carbofuran but also aids in developing monitoring strategies for environmental pesticide pollution.

Materials and Methods

I.**Experimental Organism**- healthy adult *Clarias batrachus*, weighing between 70 and 110 grams and measuring 10 to 15 centimeters, from a certified fish hatchery in Agra, Uttar Pradesh. To help them adjust, the fish were kept in aerated, dechlorinated tap water for 15 days under controlled lab conditions (with a temperature of 25 ± 2°C, pH of 7.2 ± 0.2, dissolved oxygen levels between 6.0 and 7.5 mg/L, and a light cycle of 12 hours of light followed by 12 hours of darkness). Throughout the acclimation period and the experiment, the fish were fed a commercial fish pellet diet once a day and they were fasted for 24 hours before sampling.

II.Experimental Design

Fish were divided into four groups:

(A) Control group (no pesticide)

(B) Acute Carbofuran Treated Group (I)

(C)Sub-Acute Carbofuran Treated Groups (II-IV)

(D) Sub-Chronic Carbofuran Treated Groups (V-IV)

The exposure period days 1, 7, 15, 30, 45, and 60. Throughout this time, we ensured that the water quality parameters were consistently maintained, and that the concentrations of carbofuran were refreshed daily.

III.Sample Collection and Biochemical Analysis

During each interval (on the 1st, 7th, 15th, 30th, 45th, and 60th days), fish were anesthetized using MS-222 (tricaine methanesulfonate, at a dose of 100 mg/L). Then collected blood samples by puncturing the caudal vein with 2 mL heparinized syringes after that, the blood samples were sent for centrifugation at 3,000 rpm for 10 minutes to separate the plasma, which we stored at −20°C until it was time for biochemical analysis. We also removed liver tissues, rinsed them in ice-cold saline, and used them for lipid peroxidation and enzyme analysis.

1.**Cholesterol**: Cholesterol was determined using the CHOD-PAP kit method from (Anamol Laboratories). Described by Roeschlau *et al*. (1974).

2.**Triglycerides**: Triglycerides was estimated by GPO-POD method (Anamol Laboratories) described by Schettler and Nussel (1975).

3.**High Density Lipoprotein (HDL):** was estimated by Direct Homogenous method (Beacon Diagnostics Pvt. Ltd.) described by Gordon *et al*. (1977).

4.**Low Density Lipoprotein (LDL):** Using the formula provided by Friedwald *et al*. (1972). Formula – {LDL (mg/dl) = Cholesterol – (VLDL + HDL)}

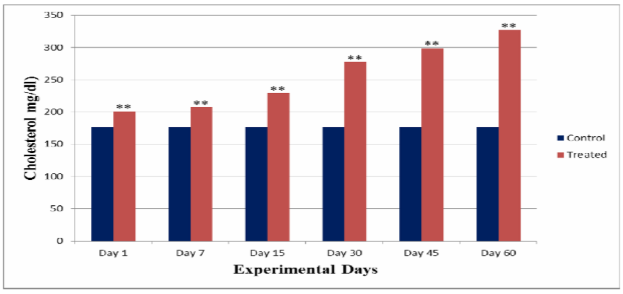
Result and Discussion

The current study took a deep dive into how carbofuran a widely used carbamate pesticide affects the lipid profile of the freshwater fish *Clarias batrachus* over six different time points (1, 7, 15, 30, 45, and 60 days). We measured lipid parameters like total cholesterol, triglycerides, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) to gauge the level of stress on the liver and any metabolic disruptions. The results showed a significant difference ($p < 0.05$) in all lipid parameters for the groups treated with carbofuran compared to the control groups, clearly highlighting the pesticide's toxic impact on liver function and lipid balance. As exposure time increased, we consistently noted a significant rise in total cholesterol and triglyceride levels. This increase suggests that lipid metabolism is being impaired, which is often linked to liver damage caused by pesticides (Gül *et al*., 2004). Since the liver plays a key role in both synthesizing and breaking down cholesterol, any harm to liver tissue can throw off the normal processes that regulate lipids (Pandey *et al*., 2003). The elevated serum triglycerides further back up the idea that fatty acid oxidation is being inhibited or that lipoprotein lipase activity is compromised, both of which can result from pesticide toxicity (Tilak *et al*., 2001). In contrast, HDL levels which play a crucial role in reverse cholesterol transport—demonstrated a steady decline throughout the exposure period. A drop in HDL often indicates hepatic dysfunction and oxidative stress, pointing to the liver's dwindling ability to handle lipid overload (Reddy & Yellamma, 1991). At the same time, LDL levels rose significantly, signaling issues with lipid clearance and serving as a sign of lipid peroxidation, a process worsened by the reactive oxygen species (ROS) created during carbofuran metabolism (Oruç *et al*., 2004). The changes we've seen in lipid profile parameters suggest that carbofuran causes hepatotoxicity in *Clarias batrachus*, influenced by both the dose and the duration of exposure. These biochemical markers can be incredibly useful for assessing environmental risks and monitoring pesticide pollution in aquatic environments. This study highlights the serious ecological threat that carbofuran poses and emphasizes the need for stricter regulations on pesticide use to avoid long-term harm to non-target aquatic species.

Cholesterol

In the control fish, cholesterol levels were found to be between 161 and 191 mg/dl, with mean of 176.60 ± 3.17 mg/dl. After treatment with carbofuran, we noticed the steady increase in cholesterol levels can be linked to issues like impaired lipid metabolism, liver problems, and changes in bile secretion, all sparked by carbofuran-induced liver toxicity. High serum cholesterol levels are recognized as a key indicator of liver damage and disruptions in lipid transport (Gül *et al*., 2004; Pandey *et al*., 2003).

•1 day: 190–210 mg/dl (200.50 ± 2.15 mg/dl)
•7 days: 193–219 mg/dl (207.50 ± 2.43 mg/dl)
•15 days: 217–239 mg/dl (229.60 ± 2.16 mg/dl)
•30 days: 263–290 mg/dl (277.70 ± 3.03 mg/dl)
•45 days: 285–318 mg/dl (298.40 ± 2.92 mg/dl)
•60 days: 314–336 mg/dl (327.20 ± 2.12 mg/dl)

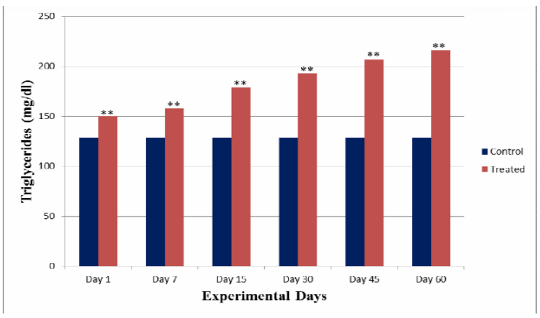


Effect of Carbofuran on Cholesterol (mg/dl) in *Clarias batrachus* (Linnaeus) after 1, 7, 15, 30, 45 and 60 days Exposure

Triglycerides

The triglyceride levels in the control group varied from 118 to 141 mg/dl, averaging at 128.70 ± 2.39 mg/dl. Interestingly, treated fish the rise in triglyceride levels seems to be connected to liver issues, where the liver's ability to break down and transfer lipids is diminished. Similar results were noted by Tilak *et al*. (2001), who found that carbamate pesticides interfere with normal lipid breakdown.

•1 day: 140–159 mg/dl (150.30 ± 1.91 mg/dl)
•7 days: 148–169 mg/dl (157.80 ± 2.53 mg/dl)
•15 days: 168–189 mg/dl (179.00 ± 2.11 mg/dl)
•30 days: 184–202 mg/dl (192.80 ± 1.60 mg/dl)
•45 days: 200–211 mg/dl (206.80 ± 1.20 mg/dl)
•60 days: 209–225 mg/dl (216.20 ± 1.60 mg/dl)

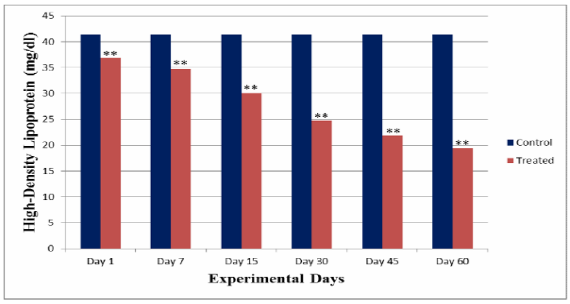


Effect of Carbofuran on Triglycerides (mg/dl) in *CLARIAS BATRACHUS* (Linnaeus) after 1, 7, 15, 30, 45 and 60 days Exposure

High Density Lipoprotein

The levels of HDL, often referred to as "good cholesterol," were noticeably lower in the treated fish when compared to the control group, which showed a range of 35–53 mg/dl (with an average of 41.30 ± 1.77 mg/dl) drop in HDL levels can indicate that the liver is under stress and that the reverse cholesterol transport system isn't functioning properly. Lower levels of HDL also mean there's a decrease in the body's anti-inflammatory and antioxidant protection (Reddy & Yellamma, 1991).

•1 day: 31–42 mg/dl (36.90 ± 0.97 mg/dl)
•7 days: 32–41 mg/dl (34.70 ± 0.90 mg/dl)
•15 days: 27–33 mg/dl (30.10 ± 0.60 mg/dl)
•30 days: 21–28 mg/dl (24.70 ± 0.87 mg/dl)
•45 days: 19–26 mg/dl (21.90 ± 0.71 mg/dl)
•60 days: 17–22 mg/dl (19.40 ± 0.48 mg/dl)



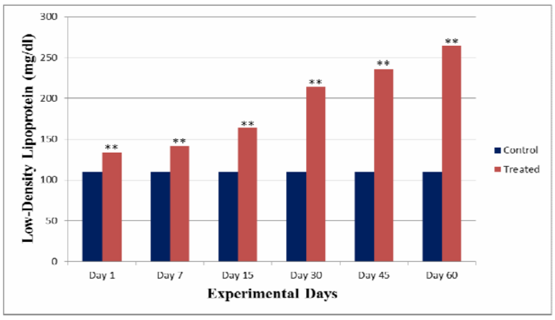
Effect of Carbofuran on High-Density Lipoprotein (mg/dl) in *Clarias batrachus* (Linnaeus) after 1, 7, 15, 30, 45 and 60 days Exposure

Low-Density Lipoprotein (LDL)

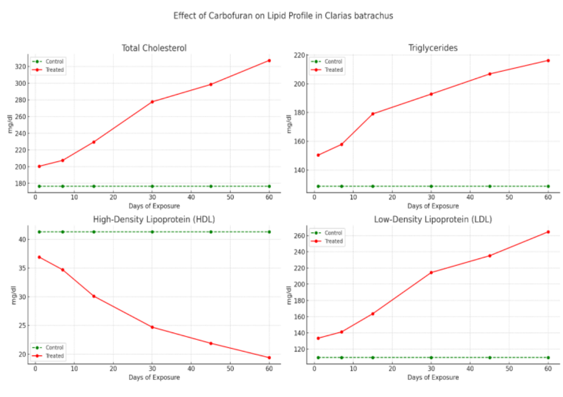
In the control fish, LDL values fell between 86.20 and 129.40 mg/dl, averaging out at 109.56 ± 3.77 mg/dl. In stark contrast, the groups exposed to carbofuran exhibited a significant rise in these values.

•1 day: 124.80–144.20 mg/dl (133.54 ± 2.10 mg/dl)
•7 days: 128.20–156.40 mg/dl (141.24 ± 2.58 mg/dl)
•15 days: 152.60–173.20 mg/dl (163.70 ± 2.18 mg/dl)
•30 days: 202.40–227.60 mg/dl (214.44 ± 2.86 mg/dl)
•45 days: 223.00–252.60 mg/dl (235.14 ± 2.68 mg/dl)
•60 days: 250.80–274.60 mg/dl (264.56 ± 2.39 mg/dl)

Increased LDL levels are often linked to liver problems and a higher risk of cardiovascular issues in vertebrates. The findings indicate that carbofuran disrupts how lipids are packaged and distributed, leading to higher LDL levels. This uptick in LDL is a significant indicator of lipotoxicity and metabolic imbalance (Kaur & Sandhu, 2014).



Effect of Carbofuran on Low-Density Lipoprotein (mg/dl) in *Clarias batrachus* (Linnaeus) after 1, 7, 15, 30, 45 and 60 days Exposure



Check out these graphs that show how Carbofuran impacts the lipid profile of *Clarias batrachus*.

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