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Histopathological Studies on Effects of Sublethal Concentrations of Lindane on the Hepatic Cells of *Clarias batrachus* based on Contaminant Concentration and Exposure Time

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ABSTRACT: The study focuses on the overall toxicological impact of lindane; a persistent organic pollutant (POP), a commonly used pesticide (Hexachlorocyclohexane) on the liver tissues of *Clarias batrachus.* The fishes were exposed to sub-lethal concentrations of 0.2 mg/L, 0.4 mg/L, and 0.6 mg/L for 24 hours and 96 hours to study the comparative effects of the toxicant over different exposure periods. Vacuolations, pyknotic nuclei, infiltration, and fat deposition in hepatic cells were visible in the liver tissues. The concentration of pesticide and exposure period were found to have an increasing effect on the alterations detected. This confirmed the toxic effects of lindane in fish, which may eventually affect humans via the food chain.

Keywords: *Clarias batrachus*, lindane, liver, histological studies, pesticides, fishes.

INTRODUCTION

Lindane, also called gamma-hexachlorocyclohexane, (HCH), gammaxene, Gammalin, inaptly glorious as benzine hexachloride (BHC), is an organochlorine chemical variant of hexachlorocyclohexane that has been used each as an agricultural chemical and as a pharmaceutical treatment for lice and scabies. Fish can assist identify the dangers associated with new chemical pollution in aquatic ecosystems because they are particularly sensitive to changes in their habitat (El-Houseiny *et al*., 2022a). Lindane is poisonous to fish that absorb it directly from water by ingesting contaminated food and bioaccumulation in their fatty acids at rates 500:1200 because of its lipophilic nature (Ortiz *et al*., 2001).

The fish under study, *Clarias batrachus* was once present abundantly in almost all marshes and swamps of Assam which has now become endangered. In this context, the present study is undertaken to investigate the impact of commonly used organochlorine pesticides lindane on the liver tissues of the fish *Clarias batrachus* to find the reason for its declining numbers in water bodies on the embankments of tea plantations and the endangered status of the fish in most of the aquatic bodies.

Lindane is poisonous to fish that absorb it directly from water by ingesting contaminated food and bioaccumulation in their fatty acids at rates 5001200 because of its lipophilic nature. Histopathological studies are conducted to assist in the establishment of causal connections between adulterant and exposure it has conjointly been evidenced to be sensitive to discovering direct effects of toxicants inside target organs of fish in laboratory trials (Sakr *et al.*, 2005; Schwaiger *et al.*, 1996). Tissue histology is considered a marker of pollution exposure and a useful technique to assess the level of pollution, particularly for sublethal effects (El-Houseiny *et al*. 2022c; Shah & Parveen 2022). The liver is taken into account as a significant storehouse organ of lipids and the point of metabolic processes in fish. Therefore, in the present study attempts have been made to assess the concentration and exposure time-based cellular alterations and damages occurring in *Clarias batrachus* through histopathological studies on the liver tissues.

MATERIALS AND METHODS

Clarius batrachus weighing 80-100g and 18-22 cm in length dipped in 0.1% potassium permanganate solution. Adequate nutrition and a proper environment were ensured.

Accordingly, the test fishes were exposed to certain concentrations of lindane of 0.2 mg/l, 0.4 mg/l, and 0.6 mg/l for a duration of 24hr and 96hr respectively in a treatment tub of 20L capacity, and covered with a mosquito net. Controlled fishes were kept in similar conditions without any treatment. The test water was changed twice every day and proper aeration was done.

Physiological saline (0.75% NaCl) was used to rinse and clean the tissue and fixed in Carnoy's fluid overnight, then dehydrated using alcohol and xylene, and embedded in paraffin wax. The tissue is then sectioned, stretched, and heat-fixed, using the standard Hematoxylin and Eosin staining procedure and observed under a high-resolution microscope.

RESULTS

Histological structure of liver of control group. The liver of the control fish *Clarius batrachus* appears as a continuous mass of hepatic cells; hepatocytes (h) with a cord-like pattern interrupted by blood vessels and sinusoids (bs). The cords of hepatocytes are arranged around the central vein (cv). The hepatocytes are massive in size and polygonal in form with centrally placed nuclei. The hepatocytes have homogenous eosinophilic cytoplasm. The sinusoids are seen as communicating channels occupied by blood cells with Kupffer cells (kc).

Histopathology of the liver after 24 hours

Treated with 0.2 mg/l of lindane. After 24 hours of exposure, hepatic parenchymatous cells exhibit mild fatty change (mFC). The polygonal shape of hepatocytes appeared to be distorted (DH) due to cellular degeneration (Plate 1b)

Treated with 0.4 mg/l of lindane. Progressive degenerative changes of hepatocytes led to the distortion of the hepatic parenchymatous cells (DH), Vacuolation(V) and damage of the membrane of the liver cells, and marked loss of intracellular connective tissue (Plate 1c).

Treated with 0.6 mg/l of lindane. The damage of the cell membrane (dCM) of hepatocytes and loss of intercellular connective tissue (arrowhead) led to massive degenerative changes in the histological structure of the liver (Plate 1d).

Histopathology of the liver after 96 hours

Treated with 0.2 mg/l of lindane. Degenerative changes in hepatocytes led to the distortion of the hepatic parenchymatous cells. Vacuolation and damage of the membrane (DCM) of the hepatocytes and marked loss of intercellular connective tissue (ICT) led to massive degenerative changes in the histological architecture of the liver (Plate 1f)

Treated with 0.4 mg/l of lindane. All the changes are found to be persisted in the fish exposed (Plate 1g).

Treated with 0.6 mg/l of lindane. Severe marked changes were observed in the histology of the liver in the group exposed to 0.6 mg/l concentration of lindane. The histology of the liver appeared to be heavily altered due to the distortion of the hepatic cords and connective tissue. Massive infiltration and deposition of fats (FD) in the hepatic cell accompanied by vacuolation (V) and pyknosis of the nuclei completely altered the architecture of the hepatic parenchymatous cells (Plate 1h)

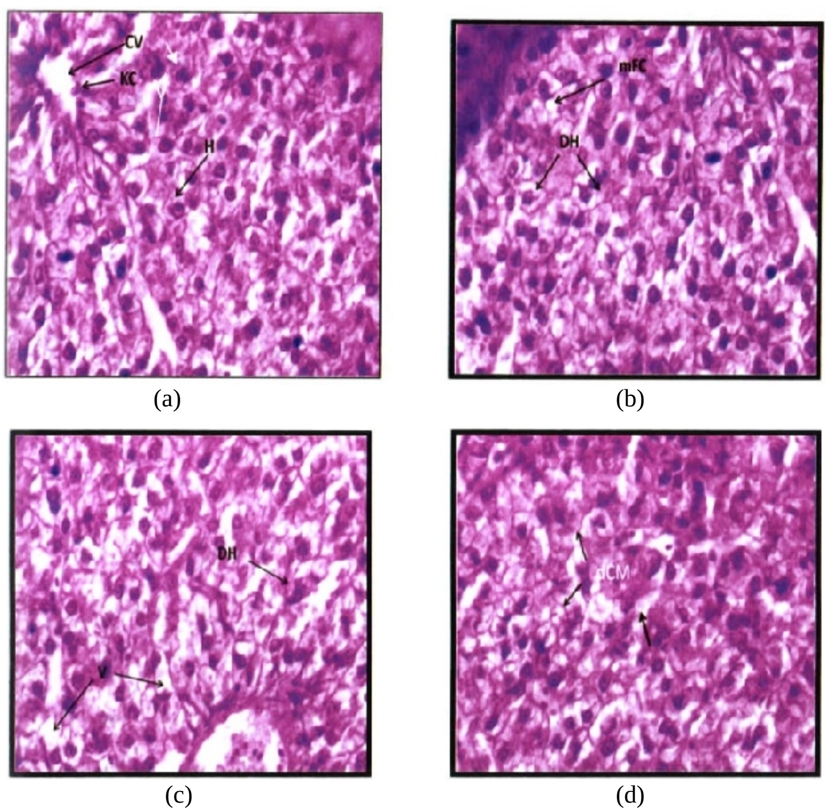
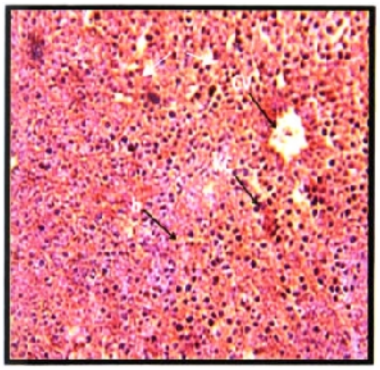


Plate 1. Photomicrographs of the liver of *Clarias batrachus* **(a)** Control, Showing hepatocyte (H) and nucleus (N), Kupffer cell (KC) and sinusoid (S), **(b)** Photomicrograph of treated (0.2mg/l) after 24 hours of exposure showing mils fatty change of hepatic parenchymatous cell (arrow), distorted polygonal shape of hepatocytes (arrow). **(c)** Photomicrograph of treated (0.4mg/l) after 24 hours of exposure showing vacuolation (V) and a complete distortion of hepatic parenchymatous cells (arrow) **(d)** Photomicrograph of treated (0.6mg/l) after 24 hours of exposure showing damage of cell membrane of hepatocytes (arrowhead) and loss of intercellular connective tissue (arrow) x400.



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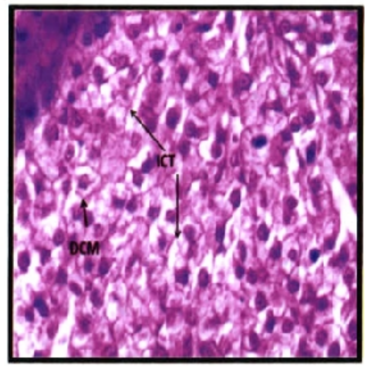

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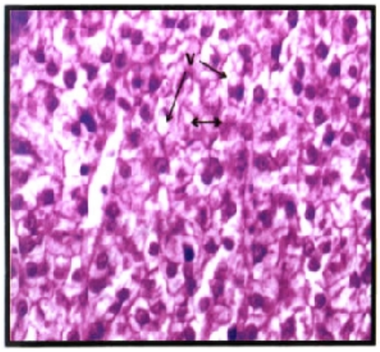

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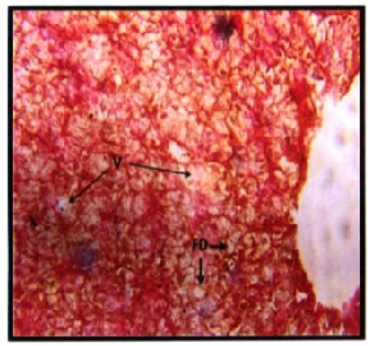

(h)

Plate 1. Photomicrograph of the liver of *Clarias batrachus* **(e)** Control, showing hepatocyte (H) and nucleus (N), Kupffer cell (KC), and sinusoid (S) x200. **(f)** Photomicrograph of treated (0.2mg/l) after 96 hours of exposure showing damage of cell membrane of hepatocytes (dH) and loss of intercellular connective tissue (arrow) x400**. (g)** Photomicrograph of treated (0.4mg/l) after 96 hours of exposure showing vacuolation (V) and a complete distortion of the hepatic parenchymatous cell (arrow), showing damage of cell membrane of hepatocytes (arrowhead) and loss of intercellular connective tissue (arrow) x400. **(h)** Photomicrograph of treated (0.6mg/l) after 96 hours of exposure showing distortion of the connective tissue (arrow) and vacuolation (V) of hepatic cells, showing pyknotic nuclei (PN) and massive infiltration and deposition of fats ins the hepatic cells (arrowhead) x400.

DISCUSSION

Histopathological observations of the liver are considered to be sensitive biomonitoring to determine the effect of toxicants on fish in pesticides polluted aquatic environments (Marchand *et al*., 2009). The liver of *Clarias batrachus* in the control was found to be normal without necrosis and hepatocytes are systematically arranged but the sample of the liver from fishes treated with different sub-lethal concentrations of 0.2, 0.4, and 0.6 mg per litre for both the exposure period has showed marked degenerative changes and resulted with severe necrosis, pyknosis, hemorrhage, and vacuolation. The alterations are found to be based on an increment of concentration and time of exposure. These histopathological changes are in agreement with the findings of Rahman *et al*. (2002) for *Anabas testudineus*, *Channa punctatus*, and *Barbodes goniotus* and of Omitoyin *et al*., (1999) for grass carp and of Ghayyur *et al.* (2021) for Mrigal and of Sulekha & Mercy (2022). The degenerative changes are intensified in a higher dose and longer exposures. They include degeneration of cytoplasm in hepatocytes, atrophy, formation of vacuoles, a rupture in blood vessels, necrosis and disappearance of hepatocyte wall, disposition of hepatic cords, decrease in size of the nucleus (pyknotic) and vacuolar degeneration within the nucleus was evident.

In the present study, lindane has induced discrete histopathological changes in the liver tissues of the entire treated group. The pathological changes noticed in the liver might affect the physiological activity of the fish such as a reduction in enzyme synthesis (Sastry & Sharma 1978; Shivalingu and Jayabhaye 2021). This reduces the functional ability of the liver which indirectly affects all metabolic activities of the organism.

CONCLUSIONS

The findings imply that when lindane is persistent over time, even a small dose may result in physiological issues in fish. Pesticides shouldn't be permitted to enter the nearby wetlands, not even in trace levels. In order to determine the levels that are safe for the aquatic ecosystem and to search for environmentally friendly alternatives, more research is needed to assess the impact of lindane concentrations on the biology of various species. It has become essential to formulate stringent rules against the indiscriminate use of this pesticide. since the pesticide is present in the environment, organochlorine as a persistent organic pollutant may induce toxic effects in fish and other aquatic fauna. Consequently, there is a possibility that they may also reach humans through food chain contamination. It is, therefore a matter of great Public Health significance to regularly monitor the pesticide residues in food and humans to assess the population exposure to this pesticide.

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Conflict of Interest. None.

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