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# Histopathological Effect of Endosulfan on the Kidney of *Clarias gariepinus* (Burchell, 1822) (Siluriformes: Clariidae)

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**ABSTRACT: Pesticides are transported into aquatic ecosystems, where they enter organisms via food webs and water contact. Endosulfan is a neurotoxic organochlorine insecticide that is used to control insect pests. In the aquatic environment, organochlorides are the most common toxicant. This insecticide is particularly hazardous to fish, and its use causes disruption of the aquatic food chain. Histopathological examination of fish tissue allows for the detection of long-term damage in cells, tissues, and organs, as well as early warning signals of disease. The kidney of aquatic animals is one of the most common organ to be affected by pollutants in the water**

**Endosulfan exposure at different sub-lethal concentrations (0.215, 0.43, 0.86µg/l) for different time intervals (5,10,15 days) resulted in profound histopathological changes in the liver, such as necrosis, vacuolization, damaged hematopoietic tissue, constriction and aggregation of renal cells, and aggregation of hematopoietic tissue. There is a corresponding increase in the degeneration and destruction of the glomeruli, convoluted tubule, renal tubule, and renal epithelium of *Clarias gariepinus* (Burchell, 1822) kidneys with an increase in the duration and concentration of Endosulfan.**

**Keywords:** Endosulfan, Histopathology, Insecticide, Kidney, Organochlorides.

## INTRODUCTION

Pesticides are commonly found in aquatic habitats including streams, rivers, and ponds at varying concentrations as they are widely used to increase crop yields by controlling pest species. Surface runoff from application sites, direct overspray, drift, atmospheric transfer, individual misuse, and improper disposal are reasons pesticides end up in water bodies. Pesticides are transported into aquatic ecosystems, where they enter organisms via food webs and water contact. As a result, the health of aquatic ecosystems is compromised because they serve as the ultimate sink for pesticides.

Endosulfan is a neurotoxic organochloride insecticide from the cyclodiene family causes DNA strand breaks and not only alters the damage response mechanism in cells, but also hindering DNA strand repair. It can even be hazardous to non-target organisms like fish, altering their physiology, metabolism, behaviour and fecundity, ultimately endangering the population's survival (Tripathi & Verma, 2004; Altinok & Capkin, 2007; Charjan & Kulkarni, 2013; Bhuvaneshwari *et al.,* 2015; Nordin *et al.,* 2018; Islam *et al.,* 2021). Histopathological studies are a useful indicator for environmental pollution because they distinguish between control and test groups and have been used to assess the health of pesticide-exposed fish (Akhter & Saha, 2013; Biuki *et al.,* 2013; Sharma & Jindal, 2020).

The effects of Endosulfan and other contaminants, such as plastics, on many aquatic organisms have been well documented (Kamble & Londhe, 2010; Ganeshwade, 2011; Nordin *et al.,* 2018; Albano *et al.,* 2021). The acute toxicity of Endosulfan to freshwater organisms was investigated by Gopal *et al.,* (1981) and the results showed that frog tadpoles are more vulnerable to Endosulfan than aquatic insect nymphs and catfishes. Mane and Muley (1984) observed dose-related behavioural alterations and mortality in two freshwater bivalve molluscs as a result of acute Endosulfan 35EC (Endocel) toxicity. Jones *et al.,* (2009) found Endosulfan to be extremely harmful to nine different species of tadpoles.

One of the first organs to be damaged by pollutants in the water is the kidney (Thophon *et al.,* 2003; Singh *et al.,* 2020). Kidney is sensitive to the presence of hazardous compounds entering the body as it is a vital organ in the body and maintaining homeostasis requires perfect kidney function. Histopathological examinations of kidney help in determining the toxin's impact. The goal of this study was to show that sublethal dosages of the pesticide Endosulfan have a far-reaching histological effect on the kidney of the African catfish *Clarias gariepinus* (Burchell, 1822).

## MATERIAL AND METHODS

*Clarias gariepinus* spawns weighing 12-13 gm and length of 10-11 cm were collected from local fish

market and brought live to the laboratory. The fishes were reared in tank of 100-liter capacity. The fishes were acclimatized under laboratory conditions for 15 days and were fed with fish food at every 24 h interval. After 15 days of acclimatization, the fishes were treated with Endosulfan 35% EC (Endocel).

*A. Chronic Toxicity Studies*

Chronic Toxicity measures long-term effects of exposure (typically 21-28 days). Sub lethal or safe level concentrations were derived from 96h $LC_{50}$ (APHA, 1992).

In the present study the 96 h $LC_{50}$ value of Endosulfan in *Clarias gariepinus*, was found to be 4.355µg/l with a 95% confidence limit ranging from 3.428µg/l (lower confidence limit) to 5.651µg/l (upper confidence limit). $LC_{50}$ values of 24, 48 and 72 h of Endosulfan in *Clarias gariepinus* are 5.912µg/l, 5.459µg/l, 4.927µg/l respectively. Chi-square test showed that the calculated values were less than the table values and is significant ($p<0.05$). The kidney tissue from each fish sample were dissected out after the fixed period of treatment, fixed in Bouin's fluid for 24 hours and processed for Delafield's Haematoxylin – Eosin staining as per the method described by Humason (1962).

## RESULT

The kidney of *Clarias gariepinus* exposed to Endosulfan exhibited pathological alterations at sublethal dosages of the insecticide. Disorganization of tissue, reduction in renal cells, damaged haematological tissue damage and renal tissue necrosis were observed in kidney tissue exposed to 0.215µg/l Endosulfan for 5 days. Changes including vacuolated glandular epithelium, swelling of renal tubules, enlarged intercellular spaces, and damaged renal epithelium were detected in tissues exposed to 0.43µg/l Endosulfan for 5 days. Adrenal tubule dilatation and glomerulus deformation could be observed in fish treated with 0.86µg/l Endosulfan (Figs. 1-4).

Glomerular oedema, vacuole formation, lymphocyte hypertrophy, renal tubule distortion, local vacuolation in tissue, bleeding, reduced glomeruli and hematopoietic tissue accumulation was seen in kidney tissue subjected to 0.215µg/l Endosulfan for 10 days. Renal tubular separation, increased sinusoids, renal tubule deformation, extensive vacuolation, severe necrosis, and desquamation of the glomerulus epithelial layer were detected in tissues exposed to a 0.43µg/l concentration. Glomerular oedema, damaged renal tubules, local tubular degeneration, deformed renal tubule, distorted glomerulus and central canal, dilated Bowman's space, desquamation of epithelial layer, and shrunken tubular lumen could be seen in tissues exposed to 0.86µg/l concentration (Figs. 5-8).

Necrosis, severe structural damage, glomerular oedema, vacuolization, damaged hematopoietic tissue, constriction and aggregation of renal cells, renal tubule distortion, aggregation of hematopoietic tissue, and shrunken glomeruli were all observed after 15 days of exposure to 0.215µg/l Endosulfan. Necrosis, shrinkage, and degeneration of glomeruli, along with disintegration of convoluted tubule, haemorrhage, disruption of glomeruli, vacuolation of renal tubule, tubular shrinkage, desquamation of renal epithelium, loss of renal tubule, and significant damage of hematopoietic tissue were noticed in tissues treated for 15 days at 0.43µg/l concentration (Figs. 9-12).

## DISCUSSION

One of the first organs to be affected by pollutants in the water is the kidney. It is in charge of selective re-absorption in order to maintain the volume and pH of blood, bodily fluids, and erythropoiesis (Iqbal *et al.*, 2004; Nordin *et al.*, 2018; Islam *et al.*, 2021).

The kidney of *Clarias gariepinus* exposed to Endosulfan exhibits several structural changes, including severe necrosis and swelling of lymphocytes, vacuolation of glandular epithelium, swelling of renal tubule, renal tubule dilation, glomerulus distortion, enlargement of sinusoids, tissue disorganisation, exudate in tubules, tubular necrosis, reduction of renal cells and desquamation of renal epithelium.

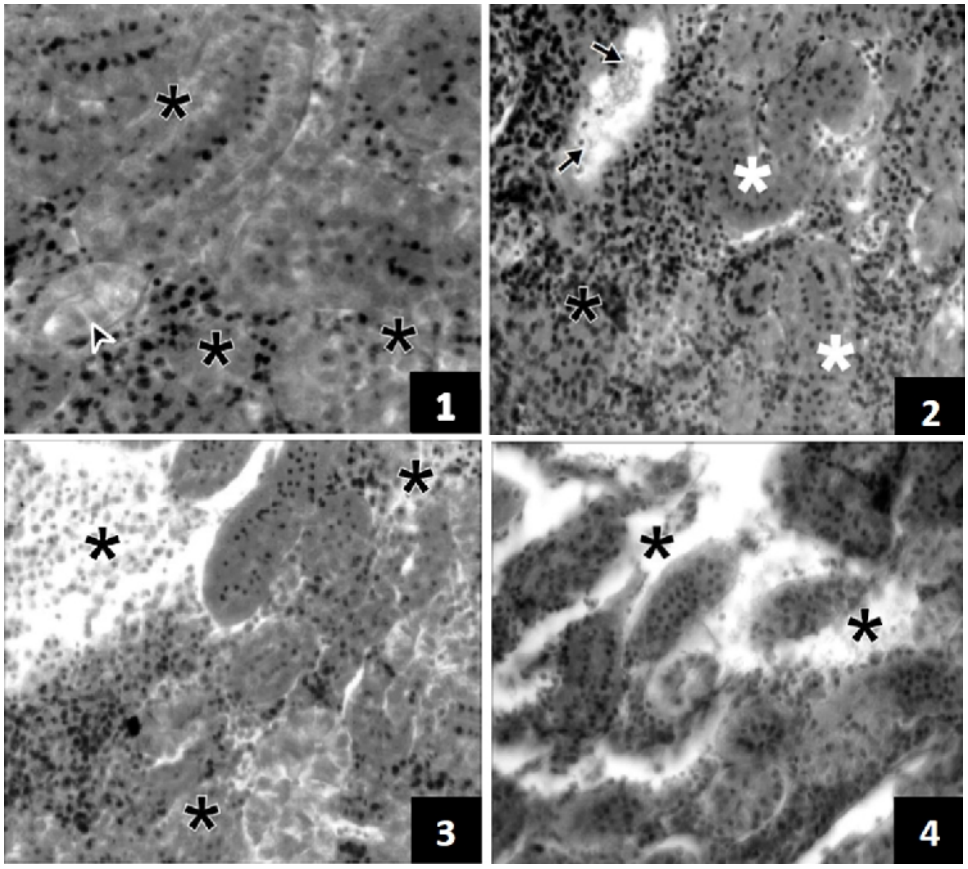
According to Takashima & Hibya (1995), tubular degeneration, dilatation of capillaries in the glomerulus, and reduction in the space of Bowman's capsule are the most prevalent abnormalities detected in the kidney of fish exposed to water pollution. Altinok & Capkin (2007) observed highly degenerative alterations in *Onycorhynchus mykiss* exposed to Methiocarb, including severe necrosis, increased sinusoids, and exudates in tubules. Butchiram *et al.*, (2009) noticed structural alterations in *Channa punctatus* kidney tissue subjected to the pesticide Alachlor, including severe necrosis, hypertrophy, swelling of renal tubules, and vacuole formation. In the kidneys of *Tilapia zillii* and *Solea vulgaris* exposed to contaminated drainage water, Mohammed (2009) observed vacuolisation and necrotic alterations.

Renal lesions are expected to be a good indicator of environmental pollution since the kidney of fish receives the majority of the post-branchial blood. Endosulfan exposure caused a time-dependent shrinkage and degeneration of the glomerulus in *Labeo rohita*, as well as vacuolization of epithelial cells (Indirabai *et al.*, 2010). Parikh *et al.*, (2010) observed oedema, vacuolar degeneration, and necrosis in kidney of *Oreocromis mossambicus* exposed to Dimethoate. In kidney of *Clarias gariepinus* exposed to phenol, Ibrahem (2012) identified deformed and damaged renal tubules. The most prevalent histopathological effect of various pesticides in the kidney of *Cyprinus carpio* was glomeruli congestion and vacuolization (Salim & Majeed, 2014). *Ctenopharyngodon idella* kidney tissue exposed to Deltamethrin exhibited structural alterations such as severe necrosis and vacuole formation (Srinivasrao *et al.*, 2018).

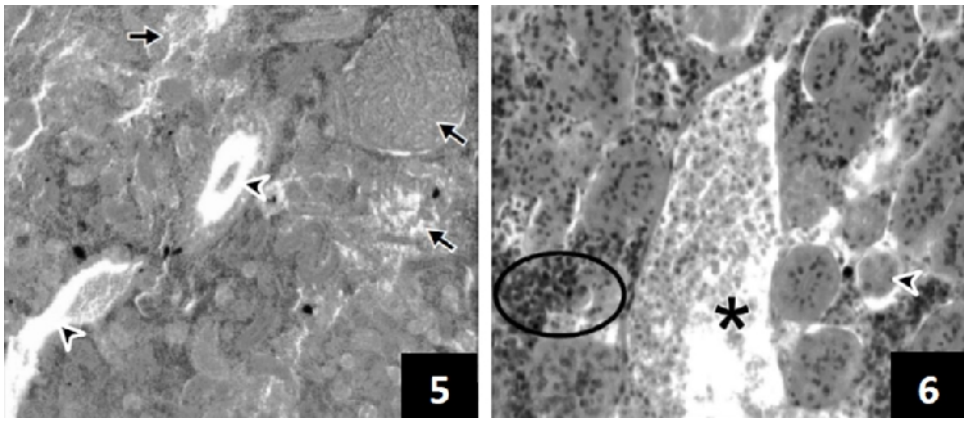
Tayel *et al.* (2014) noticed severe renal disruption, haemorrhage, and hemosiderosis in the kidneys of fish *Mugil cephalus* and *Mugil capito* exposed to untreated industrial, domestic, and agricultural drainage water, while Dhevakrishnan and Zaman (2012) observed tubular shrinkage, degeneration of tubular epithelium, atrophy of renal tubules, and formation of inter-cellular

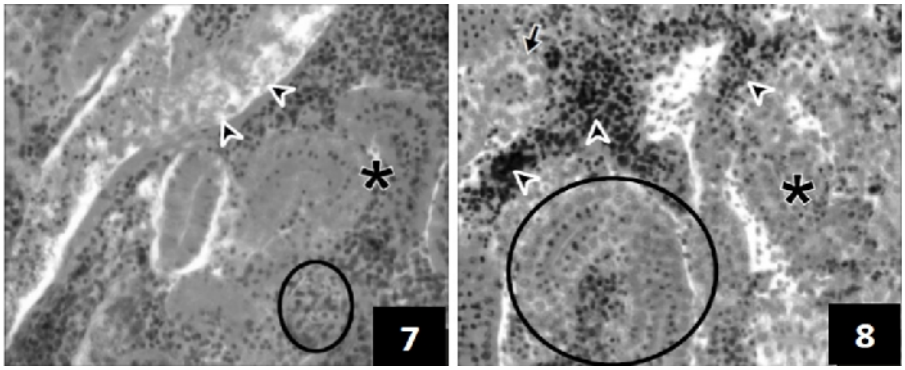
spaces in *Labeo rohita. Clarias batrachus* exposed to Endosulfan causes a significant increase in creatinine levels due to glomerulonephritis, which leads to renal failure (Singh *et al.*, 2020). These pathological changes are consistent with the histopathological findings of kidney tissue in the current study, demonstrating that Endosulfan has a significant impact on the kidneys of the catfish *Clarias gariepinus*.

The aquatic ecosystem not only provides habitat to various organisms (both plant and animal) but also is one of the main providers of human food. In order to understand, comprehend and find methods to stop the deterioration of this bio system, similar pattern of work should be undertaken to study the effect of the 21st century pollutants like metalloids, pharmaceuticals waste, engineered nanoparticle waste and microplastics (Amoatey & Mahad, 2019).

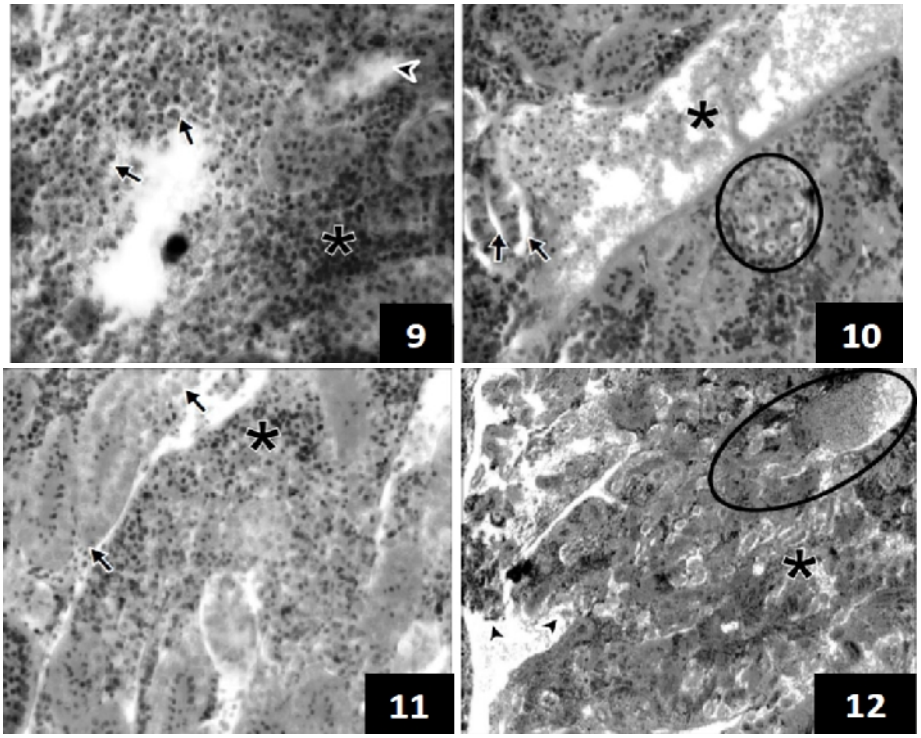



**Figs. 1-4.** Section of kidney of control and treated fish, *Clarias gariepinus* (Haematoxyline- Eosine, X200). **Fig. 1.** Section of kidney of control fish, *Clarias gariepinus* showing normal tissue vacuoles (arrowhead) and normal organization of tissue cells (asterix). **Fig. 2.** Fish exposed to 0.215µg/l Endosulfan for 5 days showing distorted renal tubules (white asterix), accumulation of hematopoietic tissue (asterix) and necrosis (arrows). **Fig. 3.** Fish exposed to 0.215µg/l Endosulfan for 5 days showing damage of renal tissue (asterix). **Fig. 4.** Fish exposed to 0.43µg/l Endosulfan for 5 days showing increased inter-cellular spaces (asterix) and extreme vacuolation.






**Figs. 5-8.** Section of kidney of treated fish,*Clarias gariepinus* (Haematoxyline- Eosine, X200). **Fig. 5.** *Clarias gariepinus* fish exposed to 0.86µg/l Endosulfan for 5 days showing severe accumulation of hematopoietic tissue (arrows) and vacuolation (arrowheads). **Fig. 6.** Fish exposed to 0.215µg/l Endosulfan for 10 days showing hemorrhage (asterix), shrunken glomeruli (arrowhead) and accumulation of hematopoietic tissue (encircled). **Fig. 7.** Fish exposed to 0.43µg/l Endosulfan for 10 days showing severe necrosis (arrowheads), distorted renal tubules (asterix) desquamation of glomerulus epithelial layer (encircled). **Fig. 8.** *Clarias gariepinus* fish exposed to 0.86µg/l Endosulfan for 10 days showing accumulation of hematopoietic tissue (arrowheads), focal tubular degeneration (asterix), contorted renal tubules (encircled) and distorted glomerulus (arrow).




**Figs. 9-12.** Section of kidney of treated fish,*Clarias gariepinus* (Haematoxyline- Eosine, X200). **Fig. 9.** Fish exposed to 0.215µg/l Endosulfan for 15 days showing vacuolization (arrowhead), damaged hematopoietic tissue (arrows) and constriction and aggregation of cells (asterix). **Fig. 10.** Fish exposed to 0.43µg/l Endosulfan for 15 days showing hemorrhage (asterix), disorganization of glomeruli (encircled) and vacuolation of renal tubules (arrows). **Fig. 11.** Fish exposed to 0.43µg/l Endosulfan for 15 days showing loss of renal tubules and damaged (arrows) and severely damaged hematopoietic tissue (asterix). **Fig. 12.** Fish exposed to 0.86µg/l Endosulfan for 15 days showing balloon necrosis (encircled), distorted renal tubules (asterix) and desquamation of renal epithelium (arrowheads).

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