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# Histopathological effect of endosulfan on the muscles 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. Given the significance of fish in the food chain, studies on the impacts of pollutants on fish are crucial in determining the consequences of pollutants on human health. For the management of insect pests, Endosulfan is an organochlorine insecticide that is neurotoxic. Organochlorides are the most prevalent toxin in the aquatic environment. The aquatic food chain is disrupted by the application of this insecticide, which is particularly dangerous to fish. Fish tissue can be examined histopathologically to identify disease-related early warning signs as well as long-term damage to cells, tissues, and organs.
We report here that Endosulfan exposure caused significant histopathological changes in the muscle, including muscle fibres splitting, broken myofibrils, disintegration of the muscle bundle, and gap formation in myofibrils, at various sub-lethal concentrations (0.216, 0.43, and 0.86 g/l) for various time periods (5, 10, and 15 days). With a rise in the time and concentration of Endosulfan, there is a corresponding increase in necrosis, inflammatory reactions, infiltration of blood cells, and dystrophic alterations demonstrating separation of the muscle bundle of *Clarias gariepinus* (Burchell, 1822) muscle.

**Keywords:** *Clarias gariepinus*, endosulfan, histopathological

## Introduction
Due to their widespread use in reducing pest species, pesticides are frequently discovered in aquatic ecosystems such as streams, rivers, and ponds at various concentrations. Pesticides get into water bodies through a variety of mechanisms, including surface runoff from application locations, direct overspray, drift, air transfer, individual misuse, and incorrect disposal. Pesticides are carried into aquatic habitats and enter creatures through food webs and water contact. Because aquatic ecosystems are the ultimate sink for pesticides, their health is harmed (Mohammad *et al.*, 2019) [18].
Endosulfan is a neurotoxic organochloride insecticide from the cyclodiene family that damages DNA strands and interferes with DNA strand repair in addition to altering cells' damage response mechanisms. Even non-target creatures like fish may be at risk since it may change their physiology, metabolism, behaviour, and fecundity, compromising the survival of the population (Altinok & Capkin, 2007; Islam *et al.*, 2021) [3, 10]. Because they distinguish between control and test groups and have been used to evaluate the health of fish exposed to pesticides, histopathological examinations are an effective indicator of environmental pollution (Akhter & Saha, 2013; Biuki *et al.*, 2013; Sharma & Jindal, 2020) [1, 4, 22].
Endosulfan and other pollutants, such as plastics, have a well-documented negative impact on a variety of aquatic creatures (Ganeshwade, 2011; Albano *et al.*, 2021; Kuriakose *et al*., 2022; Verma *et al*., 2022a, b) [6, 25]. Gopal *et al.*, (1981) examined the acute toxicity of Endosulfan to freshwater species, and the findings indicated that frog tadpoles are more susceptible to Endosulfan than aquatic bug nymphs and catfishes. Acute Endosulfan 35EC (Endocel) toxicity was seen by Mane and Muley (1984) [16] to cause dose-related behavioural changes and mortality in two freshwater bivalve molluscs. Endosulfan was discovered to be severely hazardous to nine different species of tadpoles by Jones *et al.*, in 2009. Parikh *et al.*, (2010) [12, 19] also reported moderate to severe alterations in muscles of freshwater fish *Oreocromis mossambicus* treated with Dimethoate. Because of its high protein, mineral, and unsaturated fatty acid content as well as low fat content, fish muscle is a vital and important component of human diet that has been shown to have cardiac protective effects. Control group muscle displayed typical architecture, consisting of elongated muscle fibres joined by connective tissues and having a spherical nucleus (Sumi & Chitra, 2017) [23].

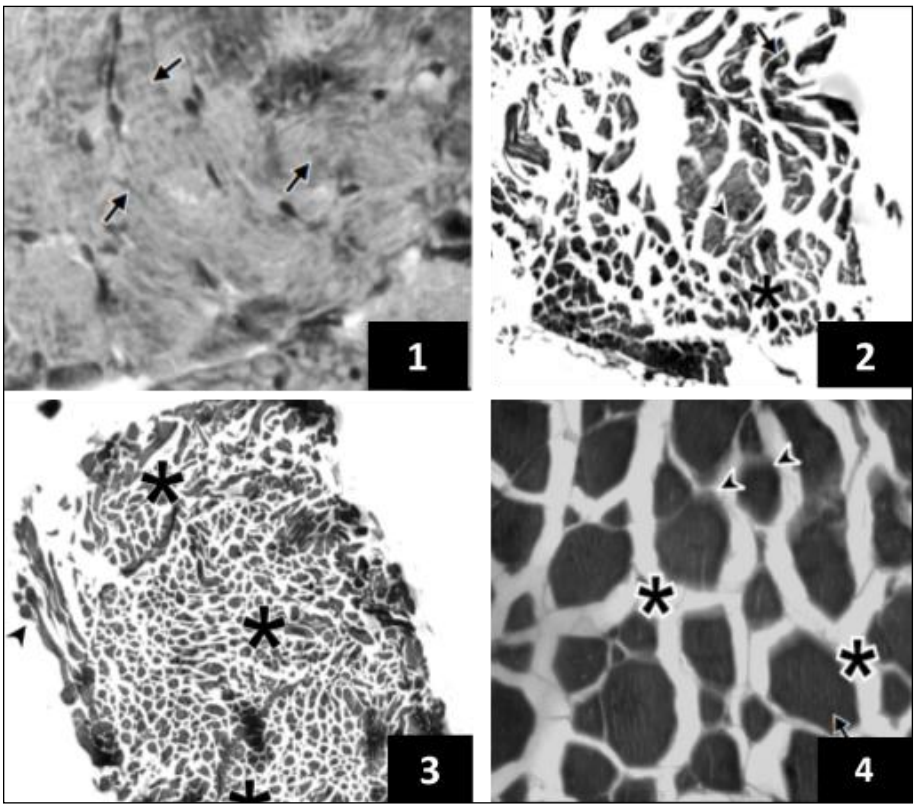
## Material and Methods
*Clarias gariepinus* spawns weighing 12-13 gm and length of 10-11 cm were collected from local fish market and brought live to the laboratory. The fishes were reared in tank of 100-liter capacity. The fishes were acclimatized under laboratory conditions for 15 days and were fed with fish food at every 24 h interval. After 15 days of acclimatization, the fishes were treated with Endosulfan 35% EC (Endocel).

## 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

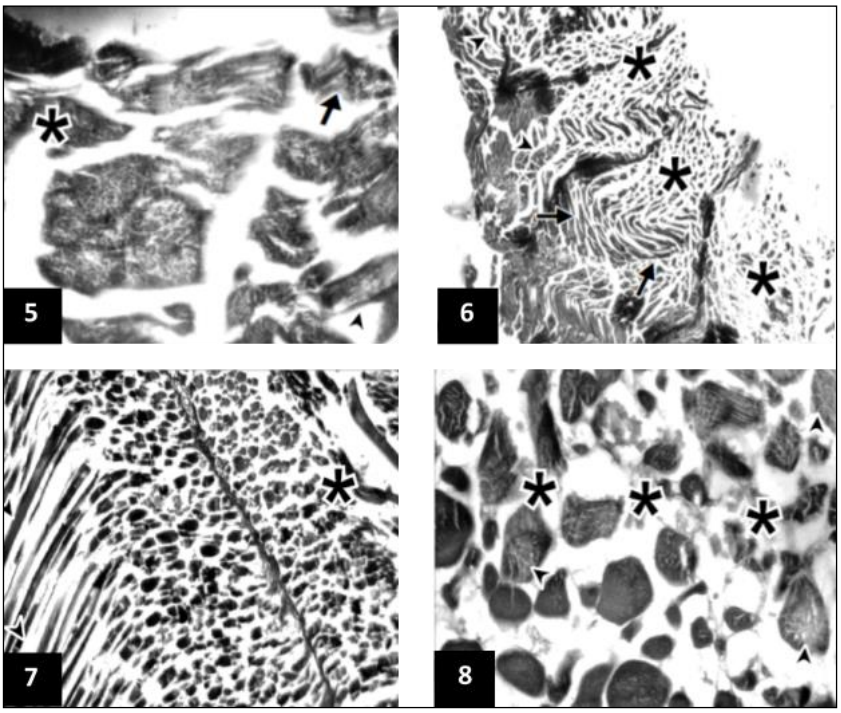
24, 48 and 72 h of Endosulfan in *Clarias gariepinus* are 5.912µg/l, 5.459µg/l, 4.927µg/l respectively. Chi-square test showed that the calculated values were less than the table values and is significant ($p<0.05$). The kidney tissue from each fish sample were dissected out after the fixed period of 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) [8].

**Result & Discussion**

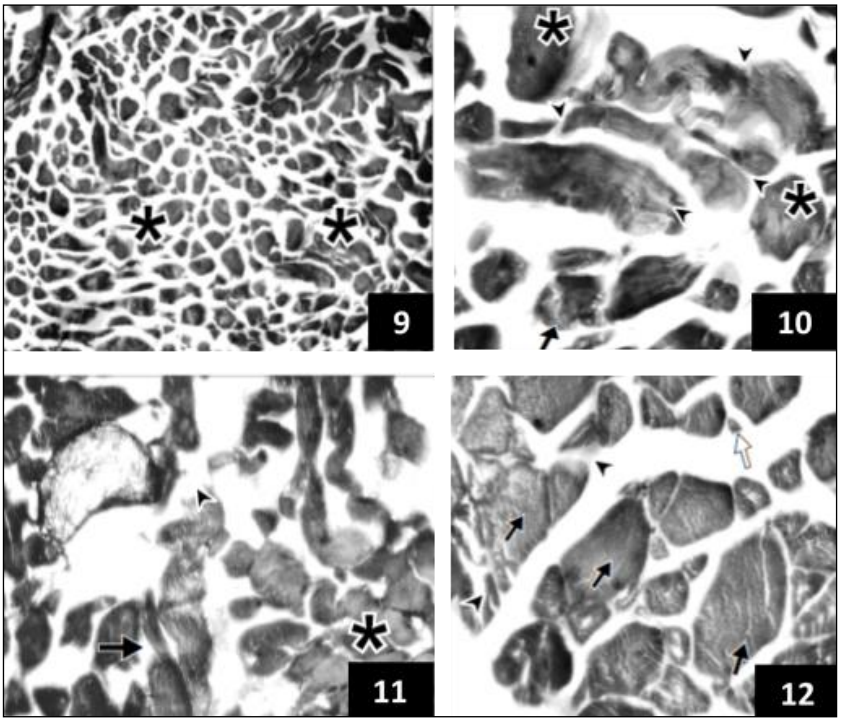
In the present work, it is observed that the muscle tissue of *Clarias gariepinus* exposed to Endosulfan exhibited histopathological changes like splitting of muscle fibres, dilation of dermal blood cells, infiltration of blood cells, congestion of dermal blood cells, muscle degradation, oedema between muscle fibres, swelling between muscle fibres, necrosis, widening of inter myofibrilar spaces leading to disintegration of myofibrils, degeneration of muscle bundle, gap formation in myofibrils, inflammatory responses, vacuolar degeneration, degeneration of muscle fibres and atrophy. The severity of damage is directly proportional to the time of exposure and concentration of Endosulfan. At highest concentration with maximum exposure clearly shows diverse effects on fish muscle (Figs. 1-12). Results of the present study agree with those observed by many other investigators who have studied the effects of different pollutants on fish muscles. The changes like necrosis, oedema, destruction of muscle fibres, dilation of blood vessels are in accordance with results obtained by El-Serafy *et al.,* (2005) [5] who worked on the histopathological changes induced on the muscle of *Oreochromis niloticus*. Mohammed (2009) [17] observed several histological alterations caused by in the muscles of *Tilapia zillii* and *Solea vulgaris*, including degeneration in muscle bundles with focal areas of necrosis, atrophy of muscle bundles and oedema between muscle bundles. Parikh *et al.,* (2010) [19] reported separation and degeneration of muscles, atrophy of muscle bundles and focal area, vacuolar degeneration and splitting of muscle fibres in freshwater fish *Oreocromis mossambicus* treated with Dimethoate. Ramesh & Nagarajan (2013) [20] reported markable changes in the histopathology of muscle tissue *Clarias batrachus* when exposed to untreated and treated sago effluent. Ibrahim *et al.,* (2013) [9] studied bioaccumulation of non-essential heavy metals Cadmium and Lead and their histopathological impact on muscles of *Clarias gariepinus.* Jeheshadavi *et al.,* (2014) [11] observed infiltration and inflammatory responses in fishes exposed to sublethal concentrations of various pesticides. Histopathological changes like ooedema, splitting of muscle fibres, separation of muscle fibres, necrosis and vacuolar degeneration were noticed during exposure of fish *Etroplus maculates* to sublethal concentrations of Fluben Diamide (Reethamma, 2014) [21]. Kazempoor *et al.,* (2015) [14] also observed histopathological changes like necrosis and inflammation in muscle cells of *Acantopagrus latus* due to water soluble fraction of Iranian crude oil. Kaur *et al.,* (2018) [13] studied histopathological effect of heavy metal contaminated water on muscle of *Clarias batrachus* with similar results as found in the present study.




**Figs 1-4:** Section of muscle of control and treated fish, *Clarias gariepinus*. Fig. 1. Section showing intact myofibrils of muscle tissue in the control fish (arrows) (Haematoxyline- Eosine x100). Fig. 2. Fish exposed to 0.215µg/l Endosulfan for 5 days depicting splitting of muscle bundle (asterix) and broken myofibrils (arrowheads) (Haematoxyline- Eosine x40). Fig. 3. Fish exposed to 0.43µg/l Endosulfan for 5 days showing broken myofibrils (arrow), oedema between muscle fibres (arrowheads) and disintegrated muscle fibres (asterix) (Haematoxyline- Eosine x40). Fig. 4. Fish exposed to 0.86µg/l Endosulfan for 5 days showing muscle degradation (asterix) oedema between muscle fibres (arrow) and increased intermyofibrilar spaces (arrowhead) (Haematoxyline- Eosine x120).




**Figs 5-8:** Section of muscle of treated fish, *Clarias gariepinus*. Fig. 5. Fish exposed to 0.215µg/l Endosulfan for 10 days showing intercellular oedema (arrow), broken myofibrils (asterix) and increased intermyofibrilar spaces (arrowhead) (Haematoxyline- Eosine x250). Fig. 6. Fish exposed to 0.43µg/l Endosulfan for 10 days showing increased intermyofibrilar spaces (arrows), disintegrated myofibrils (arrowheads) and muscle degradation (asterix) (Haematoxyline- Eosine x40). Fig. 7. Fish exposed to 0.86µg/l Endosulfan for 10 days showing dystrophic changes like separation of muscle bundle (asterix) and widened intermyofibrilar spaces leading to disintegration of myofibrils (arrowheads) (Haematoxyline- Eosine x40). Fig. 8. Fish exposed to 0.86µg/l Endosulfan for 10 days showing necrosis (asterix) and oedema between the muscle fibres (arrowheads) (Haematoxyline- Eosine x150).




**Figs 9-12:** Section of muscle of treated fish, *Clarias gariepinus* Fig. 9. Fish exposed to 0.215µg/l Endosulfan for 15 days showing muscle degeneration (asterix) (Haematoxyline- Eosine x100). Fig. 10. Fish exposed to 0.43µg/l Endosulfan for 15 days showing necrotic areas (arrows), increased intermyofibrilar spaces (arrowheads), swelling between muscle fibres (asterix) (Haematoxyline- Eosine x250). Fig. 11. Fish exposed to 0.43µg/l Endosulfan for 15 days showing disoriented muscle fibres (asterix), broken myofibrils (arrowheads), increased intermyofibrilar spaces (arrow) (Haematoxyline- Eosine x100). Fig. 12. Fish exposed to 0.86µg/l Endosulfan for 15 days showing disintegrated myofibrils (arrowheads), broken muscle (white arrow) and swelling in muscle fibres (arrows) (Haematoxyline- Eosine x200).

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