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Dimethoate (30%EC) induced toxicities on the tissues of the Indian major carp: *Labeo rohita* (Hamilton)

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ABSTRACT

Organophosphorus pesticides are used extensively in the agricultural fields due to their rapid biodegradability and non-persistence to control the pest but their broad spectrum of harmful effects extends far beyond the pest. The present study is aimed to investigate the effects of sub-lethal doses of Dimethoate (30%EC) on brain, gill, liver and kidney of *Labeo rohita*. 96 hr LC₅₀ is calculated by Probit Analysis and found to be 24.55 µl/L. Histological tissues are collected from both control and experimental groups after 10 days of pesticide exposure. Microtomy is used for histopathological studies and severe histological changes are found like in brain Dimethoate treatment drastically affected molecular and granular layers causing necrosis, in liver caused severe pathological lesions when compared to control, in kidney experimental group showed severe damage such as large space between tubules and tissue, shrunk, fragmented glomeruli with large Bowman's space enlarged lumen and disintegrated, vacuolated renal tubules, in gills several degenerative changes is also observed and it is evident from the findings that Dimethoate is moderately toxic to fish.

Keywords: Dimethoate (30%EC), *Labeo rohita*, 96 hr LC₅₀, histopathological studies.

1. Introduction

In aquatic environment, pesticides may cause several physiological and biochemical defects in fishes ^[1]. Pesticides at high concentrations are known to reduce the survival, growth and reproduction of fish and produce many visible effects on fish ^[2]. Different biological responses are used as biomarker to assess the toxic impacts of pesticides. Pesticide exposure causes severe alterations in the tissue biochemistry and histology of fishes ^[3, 4].

Dimethoate is an acaricide and insecticide, used against a wide range of insects, on ornamental plants, alfalfa, apples, corn, cotton, grapefruit, grapes, lemons, melons, soybeans, tangerines, tobacco, tomatoes, watermelons, wheat and other vegetables etc. It is also used as a residual wall spray in farm buildings for house flies and has been administered to livestock for control of botflies and leaches out into water bodies. Dimethoate is also an acetylcholinesterase inhibitor. On the other hand, *L. rohita* is a fast growing edible fish in North and East India with high economical importance. Thus, the objective of this study was to investigate the acute toxic effects of Dimethoate in Indian major carp *L. rohita* with emphasis in the histopathological changes in the brain, gill, liver, and kidney.

2. Materials and methods

Experiments were conducted from the month of November to February. Healthy and active *Labeo rohita* (Hamilton) fish (20±1.4 gm in weight and 12.7±0.75 cm in length) were brought from local market to laboratory carefully in oxygen filled plastic bags to avoid injury and disinfected for five minutes in 0.05% KMnO₄ solution. Then they were transferred to glass aquarium containing about 80 L dechlorinated tap water and gave sufficient oxygen supply. The fishes were acclimatized to laboratory condition for at least 14 days under 12:12 (light: dark) photoperiod and fed daily with were removed immediately to avoid fouling of water. Fishes were starved for 24 hours prior to toxicity testing and are not fed during the period of experiment.

Water quality characteristics were measured by using guidelines of APHA ^[5].

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Chlorine-free tap water having water qualities such as temperature: 26 ± 2 °C, pH: 7.2 ± 0.4 dissolved oxygen: 7.3 ± 0.2 ppm, total hardness: 233 ± 1.78 ppm.

Pilot experiments were run to determine the LC_{50} value which was $24.55 \mu\text{L/L}$ (through SPSS 16.0) for Dimethoate (Rogor 30% EC, Rallis India Ltd. Mumbai). The sub-lethal concentrations under study were $12.25 \mu\text{L/L}$ (1/2 of the 96 h LC_{50} value), $14.25 \mu\text{L/L}$, $16.25 \mu\text{L/L}$ and $18.25 \mu\text{L/L}$ of Dimethoate. At the end of 10 days of experiment fish of sub-lethal doses were decapitated and tissues were fixed in 10% Neutral Buffered Formalin for one week, then processed and embedded in paraffin ($60-62$ °C) and prepared for block preparation. Gill tissues were fixed in Davidson's Fixative for 24 hours and then in Neutral Buffered Formalin. The sections were cut at 5μ thickness and stained with hematoxylin-eosin counterstaining. The slides were studied under light microscope and photographed for histopathological effects.

3. Results

The Dimethoate treated fish showed copious mucus secretion with increasing dose concentration and the eyes appeared white and opaque. Pale and discoloured, slightly swollen gills were seen compared to their normal reddish and healthy appearance in control fish. Control fish behaved normally, fish aggregated in the bottom of aquarium. Irregular, erratic and darting swimming movements, loss of balance, drowning and change in body pigmentation became more apparent with increase in duration of exposure at all test concentrations compared to the control. They slowly became lethargic and hyperactive. With increasing dose exposure their opercular movements became least and died with their mouth opened. Similar alterations in behaviour of dimethoate exposed fish have been reported earlier in *Heteropneustes fossilis* [6, 7]. Histopathological changes are important to identify the environmental effects of pesticides. One of the advantages of using histopathological biomarkers in environmental monitoring is that this category of biomarkers allows examining specific target organs including gills, kidney and liver that are responsible for vital functions, such as respiration, excretion and the accumulation and biotransformation of xenobiotics in the fish [8].

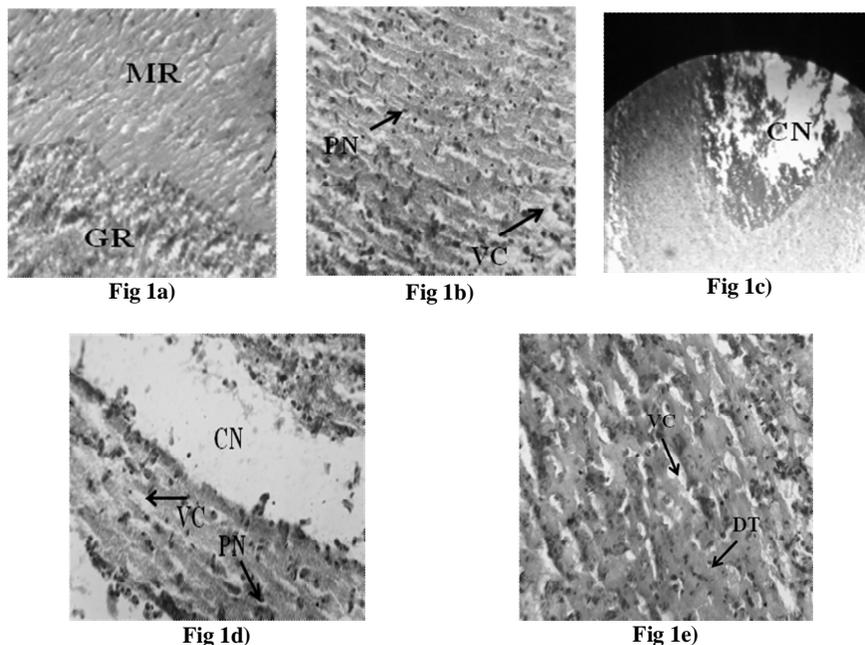
The histological observations of the control brain (Fig 1a) showed normal architecture- the molecular region, granular region and purkinje cell layer. Dimethoate treatment drastically affected molecular and granular layers causing necrosis, which is evidenced by the appearance of narrow white spaces, pycnotic nuclei (Fig 1b, d) and vacuolation in the molecular layer (Fig 1b, d, e) with increasing dose exposure. Chronic necrosis of granular region was also evident (Fig 1c, d).

Kidney from control group showed normal and systematically arranged tubules (fig 2a). Renal cortex contained normal glomeruli, Bowman's capsule and interstitium. Little or no space between muscularis and endothelium. $12.25 \mu\text{L/L}$ Dimethoate exposed showed not any marked pathological lesions. Experimental group kidney

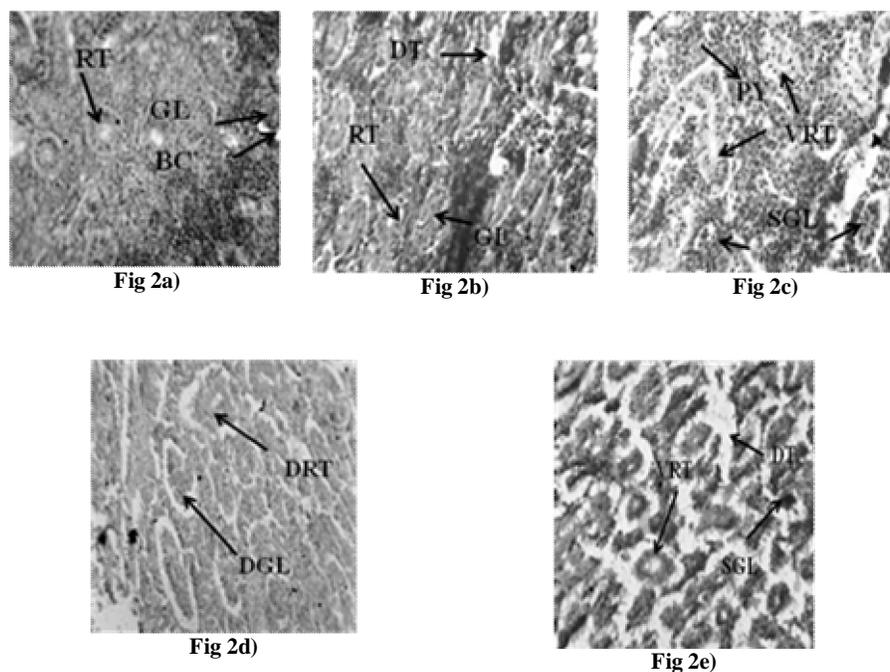
showed large space between tubules and tissue, shrunk, fragmented glomeruli with large Bowman's space (Fig 2c, d, e), enlarged lumen and disintegrated, vacuolated renal tubules (Fig 2c, d, e). Marked degenerative changes and desquamation in epithelium in some tubules were also noticed. Increased cytoplasmic granules, nuclei showed degenerating necrotic changes, hyperplasia, karyolysis, cloudy swelling, disintegration of interstitial tissue, proximal & distal convoluted tubule were moderately degenerated with increasing dose exposure. Cellular contours were not so visible at $16.25 \mu\text{L/L}$ and $18.25 \mu\text{L/L}$ exposed fish ((Fig. 2 d and e). Cuboidal epithelial cells lining the tubules showed complete vacuolation with degenerating cytoplasm and more nuclear division and renal hypertrophy.

Dimethoate exposure at different doses caused severe pathological lesions in liver tissue when compared to control (Fig 3a). The parenchyma cells- hepatocytes, biliary epithelial tissues, nuclei and non parenchyma tissues like bile ducts, arteries and veins of the liver in control groups were normal and systematically arranged. With increasing dose of exposure experimental fish liver showed loss of parenchymatous structure i.e. hepatic chord like structure, dissociated swollen hepatocytes, vacuolisation, extensively degenerated and granular cytoplasm (Fig 3b, c, d and e). Karyolysis (Fig 3d and e) and pycnosis of nuclei were also profound. Blood capillary endothelium ruptured and blood was spilled into the liver tissues (Fig 3b, c, d and e). Signs of congestion were also noticed at the sinusoid. Acute and extensive focal necrosis of hepatocytes was observed particularly at $16.25 \mu\text{L/L}$ and $18.25 \mu\text{L/L}$ exposed fish. Small spaces were appeared in between hepatic cells, damage of central veins was also observed. The cells outline becoming indistinguishable (Fig 3d and e). Some nuclei became lateral in the cell, showing variable shape and size, some with condensed chromatin that seemed to adhere the nuclear membrane.

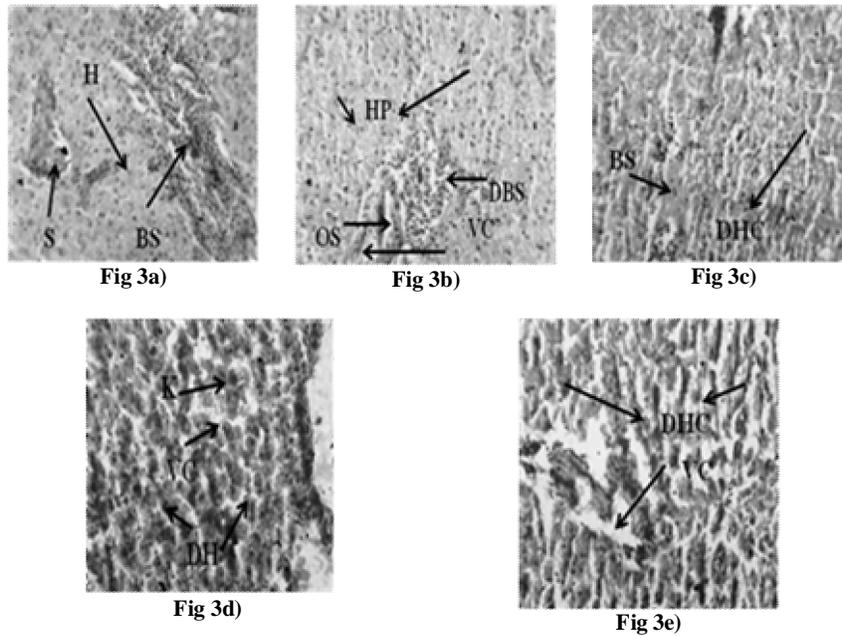
The Dimethoate treated fish gill showed marked histological changes after 10 days exposure as compared to control (fig 4a). Degenerative changes were observed in the interlamellar region. Detachment of epithelial surface in primary gill lamella from secondary lamella was observed. Swelling at the tips of the secondary gill lamellae followed by hypertrophy (Fig 4b) and marked hyperplasia. This was followed by the separation of the basement membrane, curling and fusion of adjacent gill lamellae and epithelium (Fig 4b, c, d and e). They showed reduced central axis i.e. necrotic condition which leads into interlamellar space formation (Fig. 4 b, c, d and e). So, it was evident that, acute toxicity at higher dose exposure shows extensive damage. Gill, in general, showed marked pathological changes such as bulging in the tips of primary gill lamellae, club shaped secondary gill lamellae, fusion of secondary gill filaments, proliferation of interlamellar cells, separation of epithelial layer from the central sinus of the filament and dilation of primary gill lamellae (Fig 4e), lamellar telangiectasia (Fig 4b) and hypertrophy of chloride cells.



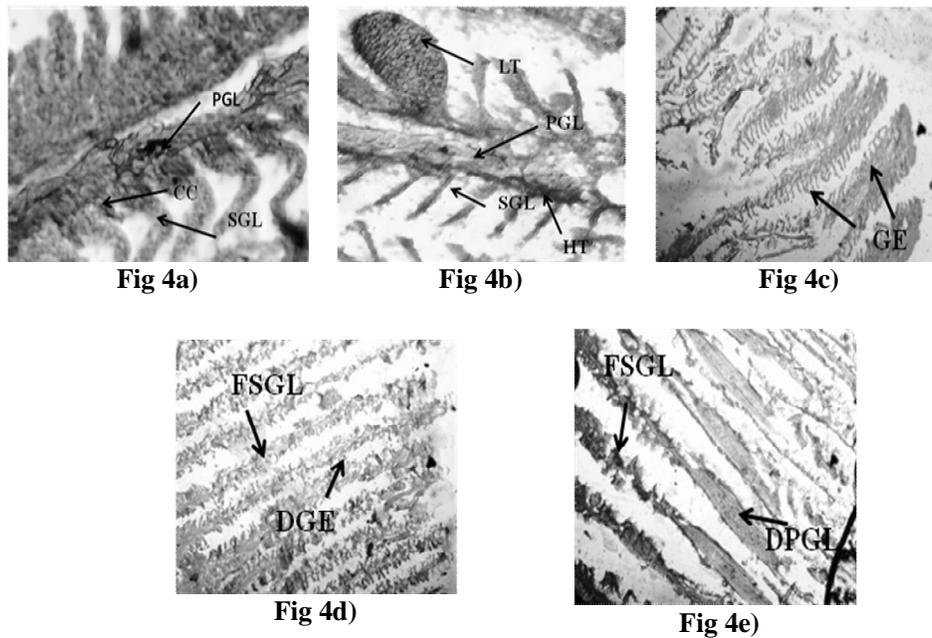
Histological sections of brain of *L. rohita*. Fig 1: Brain sections (H and E stained, X400); a- control, b, c, d and e are Dimethoate exposed for 12.25 µl/L, 14.25 µl/L, 16.25 µl/L and 18.25 µl/L respectively. MR- Molecular region, GR- Granular region, PN- Pycnotic nuclei, CN- Complete necrosis, VC- Vacuolation, DT- Degenerated tissue.



Histological sections of kidney of *L. rohita*. Fig 2: Kidney sections (H and E stained, X400); a- control, b, c, d and e are Dimethoate exposed for 12.25 µl/L, 14.25 µl/L, 16.25 µl/L and 18.25 µl/L respectively. GL- Glomerulus, BC- Bowman's capsule, RT- Renal Tubule, DT- Degenerated tissue, PY-Pycnosis, VRT- Vacuolated Renal, SGL- Shrunken Glomerulus, DRT- Degenerated Renal Tubule, DGL – Degenerated Glomerulus.



Histological sections of Liver of *L. rohita*. Fig 3: Liver sections (H and E stained, X400); a- control, b, c, d and e are Dimethoate exposed for 12.25 µl/L, 14.25 µl/L, 16.25 µl/L and 18.25 µl/L respectively. H-Hepatocytes, S- Sinus, BS-Blood Sinus, HP- Hepatic Pycnosis, DBS- Degenerated Blood Sinus, OS- Obliterated Sinus, VC- Vacuolation, DHC- Degenerated Hepatic Chords, DH- Degenerated Hepatocytes, K- Karyolysis.



Histological sections of Gill of *L. rohita*. Fig 4: Gill sections (H and E stained, X400); a- control, b, c, d and e are Dimethoate exposed for 12.25 µl/L, 14.25 µl/L, 16.25 µl/L and 18.25 µl/L respectively. CC- Chloride cell, PGL- Primary Gill Lamellae, SGL- Secondary Gill Lamellae, LT- Lamellar Telangiectasia, HT- Hypertrophy, GE- Gill Epithelium, DGE- Degenerated Gill Epithelium, FSGL-Fused Secondary Gill Lamellae, DPGL- Degenerated Primary Gill Lamellae.

4. Discussion

Intoxication of 0.35 ppm hexachlorocyclohexane to *Labeo rohita* produced mild vacuolar changes with small spaces in brain, whereas 1.73 ppm exposure showed severe necrosis [9]. Severe damage in brain cells and neural cells with broken

neural bundles were observed in 100 ppm malathion treated *Ophiocephalus punctatus* [10]. Recently, above mentioned alterations were also found in common carp exposed to atrazin and chlorpyrifos [11]. Mild vacuolar changes with empty spaces appeared due to increased concentration and

duration of zinc toxicity to *Labeo rohita* ^[12]

It is evident from the above findings that Dimethoate is moderately toxic to fish. In the present study control fishes behaved normally, on the other hand experimental fish showed erratic, daring, swimming movements. This uncoordinated behaviour may be due to inhibition of AChE activity leading to accumulation of acetylcholine in the cholinergic synapses with hyperstimulation. Severe congestion and generalised spongiosis indicating severe brain damage was also found.

Cytoplasmic granules may be formed inside the cells or the reabsorption of plasma proteins lost in the urine, indicating damage in the corpuscle ^[13]. Kidney damage was due to elimination of undetoxified toxicant molecule through urine. On the other hand, as ultra-filtration occurred in glomerulus, so extensive damage found here.

The liver is the largest organ of the body serving multiple functions. It has no direct contact with the environmental pollutants dissolved in water but due to its contact with blood, it's indirectly affected. The diffusion of OP depends on lipid solubility and the removal by the blood depends on the lipid content of the blood or special carriers ^[14]. This was the reason for some changes in the liver cell morphology being observed first close to blood vessels. Singh et al. also noticed such type of changes in common carp (*C. carpio*) after Dimethoate exposure ^[15].

Gill is the respiratory organ, so contact between gill and pesticide may cause damage of gill tissue and diffusion capacity of gill which in turn to reduced oxygen uptake ^[16]. Decrease in dissolved oxygen which was evident from our study may cause a stress and resulted in severe gill damage.

5. Acknowledgements

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