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TOXIC IMPACTS OF INSECTICIDE POLO ON HISTOPATHOLOGY OF GILL, LIVER AND KIDNEY AND GLYCOGEN CONTENTS OF A FRESHWATER FISH, *LABEO ROHITA*

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ABSTRACT
The broad spectrum insecticide Polo was used for this investigation to study the biochemical and histopathological effects in freshwater fish, *Labeo rohita*. The LC50 value of Polo was found to be 1.9ppm for 96 hrs. under laboratory conditions and 1/10 of the corresponding LC50 value was treated as sub lethal concentration. The fish showed decreased amount of glycogen content in liver and gonads (Testis and Ovary) and severe histopathological changes in gills, liver and kidney.

KEY WORDS: *Labeo rohita*, Polo, Biochemical, Histopathology.

INTRODUCTION
The widespread and indiscriminate use of chemicals for controlling the agricultural pests, many aquatic organisms like fishes, bivalves, prawns, crabs *etc*. are getting affected. The damage of different tissues and alteration in biochemical processes as well as disturbances in physiological processes were observed by these pollutants. Insecticide Polo is widely used in agriculture for controlling the crop pests. For this reason Polo was used in this investigation to study its toxic effects in fish. Acute exposure (24 to 96 hrs.) of Polo alters the normal architecture of tissues like gill, liver and kidney and decreases the glycogen contents of fish *Labeo rohita*.

MATERIALS & METHODS
The fish *Labeo rohita* were collected from Ganeshpur and Girna river dam near Chalisgaon city, Dist. Jalgaon, Maharashtra, India and were acclimatized to laboratory conditions for 15 days into 1000 liter capacity tank, previously washed with potassium permanganate and water temperature was 26.3±35degree centigrade and pH 7.0 to 7.2 maintained in aquarium. The acclimated healthy and active fishes were exposed to the acute toxicity (up to 96 hrs.) exposure to an insecticide Polo.

Toxicity assay
Group of 10 healthy and active fishes *Labeo rohita* were kept in a glass tank of de-chlorinated tap water. The fishes were treated with varying concentrations of Polo to determine the LC50 values. 1/10 of the LC50 values were taken as sub lethal concentration for the 24, 48, 72 and 96 hours of the experiment. To observe the histopathological changes, a group of individuals exposed to different concentrations as 3ppm for 24hrs. 2ppm for 48hrs. 1.5 ppm for 72hrs. and 1ppm for 96hrs. (Sub lethal concentrations are 0.3ppm for 24hrs., 0.2ppm for 48hrs., 0.15ppm for 72hrs. and 0.1ppm for 96hrs. that means 1/10 value of LC50.) All individuals in control were maintained in toxicant free de-chlorinated water in the separate tank. During this experiment mortality was recorded for 24, 48, 72 and 96hrs. respectively. After exposure and completion of treatment, *Labeo rohita* were dissected and gills, liver, gonads and kidney were gently separated. The gills, liver and kidney tissues were fixed in Bouin's fluid and were processed by routine micro technique method and blocks were prepared. Then these prepared blocks of the tissues were cut with the thickness of 6µ on microtome. The ribbons of sections were spread on slide and these slides were further processed for double staining method. After the completion of these procedures, the slides were observed under microscope. Slides were observed under oil imersion for histolopathological details. The tissues of liver, testis and ovaries were also processed for glycogen estimation by Anthrone reagent method (Dezwann and Zandee, 1972).

RESULTS & DISCUSSION
From the histopathological observations, the insecticide Polo had altered the structure of gills, liver and kidney. In fishes, the gills are the most important organ for respiration and osmoregulaton and it is the first organ which the pollutant comes into contact. Gills are the main route through which toxicants enter into the body. Control gill (fig no. 1) showed a gill arch with double row of elongated laterally projecting primary gill filament, which in turn bear leaf like projections, the secondary gill lamellae. Each secondary gill lamellae is delicate flattened structure comprising of a pair of two layered epithelial sheets supported by pillar cells which in rows occupy the whole area of secondary gill lamellae .On the basement membrane of epithelial covering, neighboring pillar cells fuse to complete the lining of lamellae and blood channels, which connect the afferent and efferent lamellar vessels.The gill of the fish *Labeo rohita* exposed to the insecticide Polo (96hrs.) (Fig no.2) had showed marked histopathological changes characterized by swelling and degradation in respiratory epithelial cells and connective tissue cells. Connective tissue cells lost their normal cellular structure.

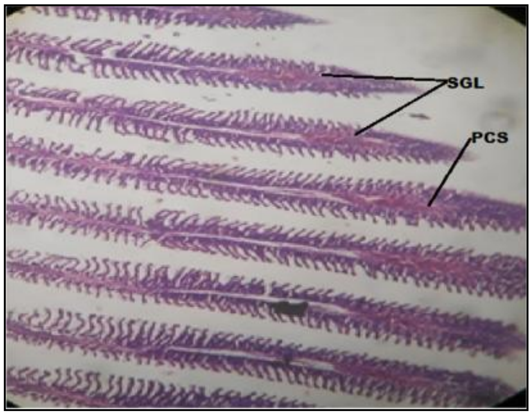


Fig.No 1
Control Gill of *Labeo rohita* at 100x
PCS – Pillar cells
SGL- Secondary gill lamellae

Fig No. 2
Polo treated Gills of *Labeo rohita* at 100x
PGL- Primary Gill lamellae
PLE – Primary lamellar epithelium

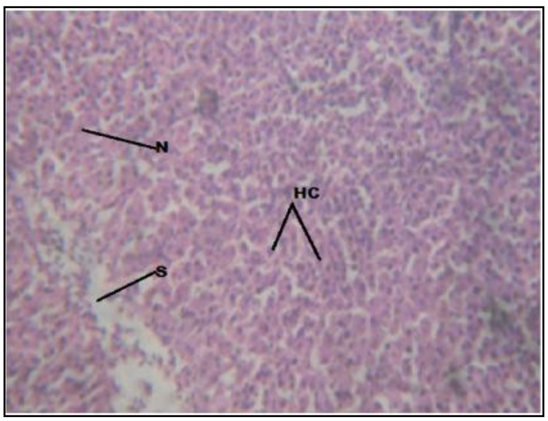


Fig.No 3
Control Liver of *Labeo rohita* at 100x
HC – Hepatocytes
N- Nucleus

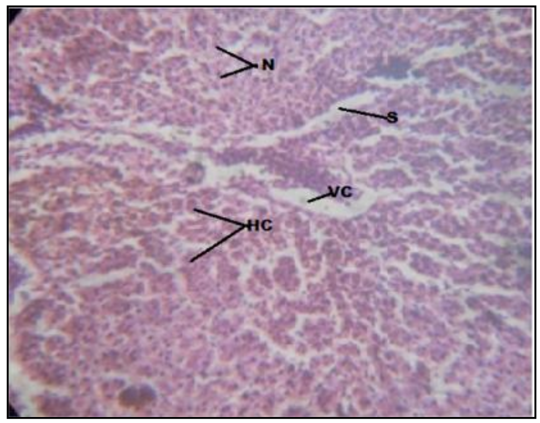


Fig.No 4
Polo treated liver of *Labeo rohita* at 100x
VC - Vacuolization
S - Sinusoid

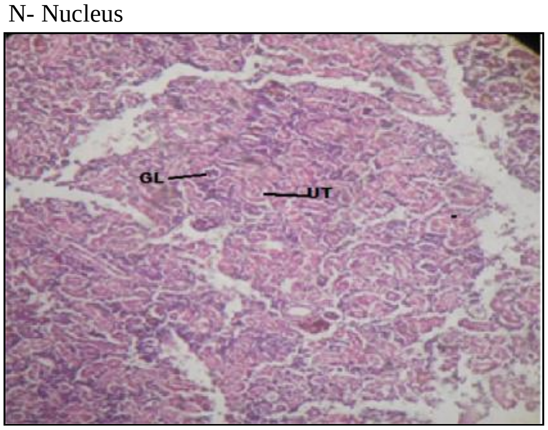


Fig.No 3
Control kidney of *Labeorohita* at 100x
UT – Uriniferous tubules
GL - Glomerulus

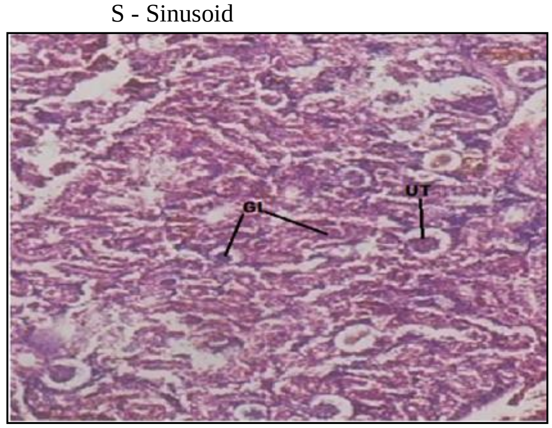


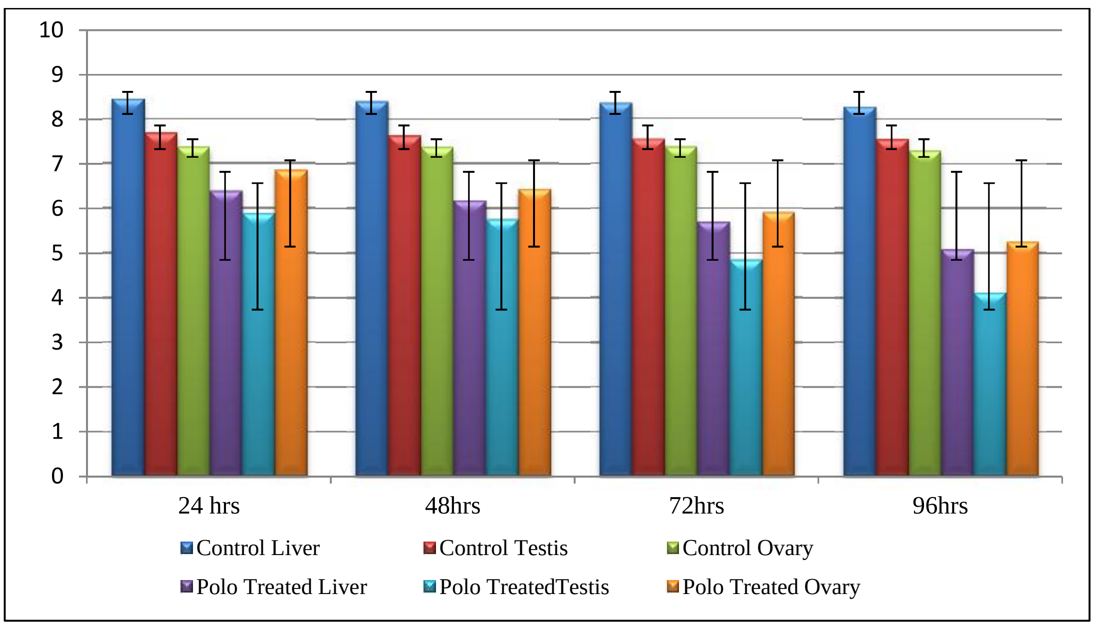
Fig.No 4
Polo treated Kidney of *Labeorohita* at 100x

The secondary gill lamellar cell walls are disappeared, gill lamellae are shortened, and necrotic changes in respiratory epithelium of gills resulting in the development of vacuoles were seen. Cytoplasms showed disintegration at greater degree because of hyperplasia of respiratory epithelial cells and reduced inter lamellar space. Severity of damage in the gills was found to be dose dependent. The liver is an important organ performing vital functions. Control liver (fig no. 3) showed large polygonal hepatocytes. These hepatocytes are separated by blood sinusoids. Each hepatocyte showed a distinct round and central nucleus with nucleoli and granular cytoplasm. Polo treated liver (fig no. 4) showed changes in its architecture. Hepatic cell diameter was changed, extensive cytoplasmic vacuolization had seen and nuclei become pyknotic and excentric. Histopathological alterations resulting from an exposure of Polo may affect the functional efficiency of the liver, leading to malfunctioning of several organ systems of the fish. Histology of control kidney (fig no. 5) of fish *Labeo rohita* showed distinct and normal size of proximal and distalconducting tubules of glomerulus with connective tissues. Whereas, Polo treated kidney (fig no. 6) showed damaged proximal and distal tubules as well as sinus appeared in connective tissues. The depletion in glycogen contents in liver and gonads after acute exposure by Polo were increased as the period of exposure increased. The maximum depletion occurred in the liver followed by gonads. The results are summarized in table no1. There was depletion in glycogen content in liver and gonad as compared to the control. Liver is the vital organ of carbohydrate metabolism. It was affected by Polo. Decrease in glycogen values in liver was noticed by K. Suneetha (2011). Carbohydrates are the primary source of energy in stress condition. Depletion of glycogen may be due to direct utilization for energy generation demand caused by pesticidal stress. Total depletion of glycogen would result in the disruption of enzymes associated with carbohydrate metabolism. Decrease in glycogen content in liver were reported by Muleyetal, (2007); Balaji and Chockalingam (1991), Amudha and Mahalingam (1999), Maruthi and Rao (2000), Mohammed A. Al. Khatani (2011), Venkatramana *et al.*, (2006) and Logaswamy *et al*. (2009).

Table- 1 Toxic impact of insecticide Polo on Glycogen contents in Liver and Gonads (testis and Ovary) of *Labeo rohita* after acute exposure.

Tissue	Treatment	Acute			
		24 hrs	48hrs	72hrs	96hrs
Liver	Control	8.4505 ±0.008246***	8.3939 ±0.010392***	8.3548 ±0.145602***	8.2829 ±0.000078***
Testis	Control	7.6851 ±0.006164***	7.6249 ±0.009165***	7.5536 ±0.008831***	7.5431 ±0.02607***
Ovary	Control	7.3768 ±0.034058***	7.3764 ±0.03286***	7.3762 ±0.02898***	7.2961 ±0.05639***
Liver	Polo	6.3913 ±0.08485***	6.1766 ±0.03065***	5.6962 ±0.02323***	5.0832 ±0.02236***
Testis	Polo	5.8921 ±0.02898***	5.7558 ±0.02449***	4.8438 ±0.04795***	4.1152 ±0.02792***
Ovary	Polo	6.8571 ±0.006928***	6.4332 ±0.006164***`	5.9102 ±0.01077***	5.2567 ±0.008296***

1) Values expressedas mg/100g of wet wt. of tissues.
2) ± indicate S.D. of five observations.
3) Values are significant at P<0.001***.



GRAPH: Variation in Glycogen content of Liver and Gonads (Testis and Ovary) of *Labeo rohita* after acute exposure to insecticide Polo

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