Altered Histology Of Kidney Of The Fresh Water Ornamental Fish, *Puntius Conchonius* (Ham.) On Exposure To Sublethal Doses Of Two Commonly Used Pesticides

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Abstract: The ornamental fish, *Rosy Barb, Puntuis conchonius* is a common aquarium fish because of its attractive colouration. The current study focused on the sensitivity of this fish to aquatic pollution. Unlike other fishes, the study animal showed high sensitivity and heavy damage to the kidney tissues that is degeneration of renal tubules, Glomerulus and Bowman's capsule. Cellular necrosis and haemotopoiesis were also observed.

***Index Terms* – *P. conchonius*, haemotopoiesis, necrosis, kidney, glomerulus, renal tubules**

I. Introduction

In India, aquaculture and agriculture are significant sectors. Systemic pesticides are widely used in agriculture, which leads to two main effects: first, the soil of the treated crops becomes contaminated, and second, residues are transferred to the aquatic environment (Bayo et al. 2016). In general, pesticides cause habitat destruction, lower food supplies, and hinder reproduction, which lowers species diversity in the animal kingdom and contributes to animal and plant population declines (Shefali et al. 2020; Zahid et al., 2019). In more than 60 countries worldwide, endosulfan ($C_9H_9Cl_6O_3S$), a chlorinated hydrocarbon insecticide, is used in agriculture to control pests (Vidal et al 2000). Another class of pesticides that are harmful to aquatic life is synthetic pyrethriods. For over thirty years, pyrethroid insecticides have been utilized in agriculture to manage insect pests in various crops. According to Cassida et al. (1998,) fenvalerate is a pyrethroid insecticide that is most frequently used on agricultural crops like cotton, paddy, jowar, maize, soyabean, tomato, lady's finger, cauliflower, tobacco, and tea. There are several ways that these agro-pesticides can infiltrate aquatic environments, including spray drift, runoff, and leaching (Shahjahan et al. in 2017). Furthermore, synthetic chemical pollutants enter the water ecosystem through agricultural sources and seep into nearby waterbodies and the groundwater table and have been shown to exert adverse effects on aquatic organisms. Fish have proved to be of significance as bioindicators of the aquatic environment so-called ecological integrity (Faggio et al. 2014a, b; Gobi et al. 2018; Bartoskova et al. 2013). Therefore, fishes are successful bioindicators. The present study is to investigate the sensitivity of the study animal to chemical pollutants in freshwater system. Histopathology provides a rapid method to detect the effects of irritants in various organs (Johnson et al 1993) . The exposure of fish to chemical contaminants is likely to induce a number of lesions in different organs (Bucke et al, 1996). Kidney (Bucher et al., 1993) is one such a suitable organ for histological examination to determine the effect of pollution. In fish, the kidney performs an important function related to electrolyte and water balance and the maintenance of a stable internal environment. Histopathological investigation of the liver and kidney has been acknowledged as an excellent tool for studying the acute toxic effects of agro-pesticides on fish, as well as essential indicators of physiological stress caused by any anthropogenic stressor (Hossain et al., 2016; Islam et al., 2019; Sumon et al., 2019; Reza et al,. 2020). The indiscriminate use of agro-pesticides is drastically rising in order to increase crop production and feed the growing human population. These pesticides have harmed fish due to their alarmingly high bioavailability in open water environments. Thus, the current study's goals were to ascertain how endosulfan and fenvalarate affected the key internal organ, the kidney of a significant freshwater fish known as Rosy barb, *P.conchonius* when subjected to various sub-lethal concentrations.

MATERIALS AND METHODS

The physico-chemical characters of the water used in the present study were estimated following standard methods described by APHA (1975). Fenvalerate is a commercial grade liquid synthetic pyrethroid (Cyano(3-phenoxyphenyl) methyl 4-chloro-2-(1-methyl-ether) benzeneacetate, fenvalerate, 20% EC) marketed by Isagro (Asia) Agrochemical Pvt.Ltd., Panoli-Mumbai, India as Fenval was used throughout the experiment. Endosulfan is a commercial grade organochlorine pesticide (6, 7, 8, 9, 10-hexachloro-1,5,5a,9a-hexahydro-6,9-methano-2,4,3-benzodi-oxithepin-3-oxide; 35% EC) marketed by Excel Crop Care Limited, Mumbai, India, as Endocel was used throughout the experiment.

Sublethal Concentrations

LC_{50} Values were determined for both the pesticides as prescribed by McLeay (1973). Probit analysis was done to derive mortality values for each pesticide. This would indicate the Log LC_{50} values of the pesticides for the experimental group exposed to a period of 6, 12 ,48 , 72 and 96 hours (Finey, 1971). After this two sublethal concentration of each pesticides namely endosulfan $1/10^{th}$ (1 x 10 -7 ppm) and $1/20^{th}$ (5 x 10 -8 ppm) and fenvalarate $1/10^{th}$ (9 x 10 -7 ppm) and $1/20^{th}$ (4.5 x 10 -7 ppm) were used for this study.

EXPERIMENTAL DESIGN

Healthy *P. conchonius* weighing 2000 ± 200 mg (live weight) were selected from stock tanks and exposed to two sublethal concentrations of each pesticide, namely endosulfan {1/10th($1x10^{-7}$ppm) and $1/20^{th}$($5 x10^{-8}$)}and fenvalerate {$1/10^{th}$ ($9x10^{-7}$ppm) and $1/20^{th}$($4.5x10^{-7}$)} dilutions of 96 hours. LC_{50} value was determined as prescribed by Mc Leay (1973). Three replications with ten individuals [healthy *P. conchonius* weighing 2000 ± 200 mg (live weight)] in each trough of 17 l capacity for each sublethal concentration of both the pesticides were maintained for 21 days. Fresh test media were supplied daily. The fish were fed ad libitum with pelleted feed of 35% protein at 10.00 hours everyday. Simultaneously a control group of 10 individuals was maintained throughout the experimental period in well water. The experimental concentrations were prepared using the same well water. After exposure for 21 days five fish from each replication of the sublethal concentrations were sacrificed to obtain the necessary tissues for histopathological studies.

Preparation of Permanent Microscopic Slides

The gill tissues were fixed in Zenker's fluid, dehydrated and embedded in paraffin following the method of Wesner (1968). Sections at 7mm thickness were prepared using rotary microtone. After deparafinizing, the slides for Histological and Histopathological observations were stained using one of the following stains:

1. Ehrlich's hematoxylin used for pathological studies as nuclear stain
2. Aqueous 0.2% Eosin Y as the cytoplasmic stain and
3. Van Gieson with Methylene blue to study connectivity tissues.

Finally the sections were mounted in DPX (Weil, 1945).

RESULTS

Histology of normal Kidney

The kidney in *P. conchonius* occupies a dorsal position in the body cavity and is placed just ventral to the vertebral column and dorsal to the alimentary canal and the gonads. The functional unit of kidney is nephron. Each nephron consists of two parts: the renal corpuscles (Malpighian body) and the renal tubule (urinary tubule). The renal corpuscle or Bowman's capsule is double layered, cup-like structure of uriniferous tubule, which contains a tuft of capillaries known as glomerulus. The renal tubule is divided into three regions: proximal convoluted segment, the intermediate and distal convoluted segments. The glomerulus and Bowman's capsule together constitute the renal or Malphigian corpuscle. The glomeruli are numerous and large in size. The renal tubules are thin and short in the neck segment, and consist of a single layer of low epithelial cells with long cilia. The proximal convoluted segment has coarse granules in the cytoplasm. The rest of the kidney is filled up with interstitial lymphoid tissue and connective tissue (Plate: 1 to 4).

Histopathological changes in Kidney exposed to fenvalerate

The structural organization of the kidney of toxicant exposed *P. conchonius* shows varying degrees of deformities. Plate 5 shows the view of a longitudinal section passing through the primary collecting tubule under higher magnification and reveals the enlargement of brush borders in it. Plate 6 exhibits the same condition when sectioned transversely. Plate 7 shows a completely disorganized glomerulus due to toxicity effect and it is filled with yellow pigmented particles.

($1/20^{th}$ LC_{50})

The extent of damage to the structure of kidney of the fishes exposed to this concentration is lesser when compared to previous concentration. Plate 9 shows a small glomerulus and plenty of stray R.B.Cs in the entire sectional view. A swollen glomerulus and R.B.Cs are also seen in another section (Plate, 10). Plate 11 shows two glomeruli very much reduced in size with abnormal structure. The renal collecting tubules have been narrowed down to the extent that the lumen is closed.

Histopathological changes in Kidney exposed to endosulfan

(1/10th LC_{50})

P. conchonius exposed to this concentration has suffered a deleterious effect. Plates 12 to 15 show very serious disintegration of kidney tissue in different fishes. However, normal glomeruli are also seen. The renal tubules have disintegrated and some appear in normal histology. A cyst like congregation surrounded by many layered cyst wall is seen in all the sections. The wall surrounds a mass of disintegrating cells. Plates 14 and 15 show very aggravated condition of cyst-like formation in the kidney owing to deleterious effect of the chemical treatment.

(1/20th LC_{50})

Plates 16 to 18 indicate the toxic effect of this chemical on the kidney of *P. conchonius*. In Plate 16, normal glomeruli and renal collecting tubules are seen at most part of the view. Some renal tubules are disintegrating due to the chemical effect. The glomerular region looks as if it is replaced by 'cyst' formation. Plate 16 also reveals the image of kidney tissue partly damaged and partly healthy. Most of the renal collecting tubules are under disintegration. A normal glomerulus and a few renal tubules in normal histology are also evident. Plate 17 shows a many layered 'cyst wall' surrounding a mass of disintegrating cells. The renal tubules appear totally collapsed.

DISCUSSION:

Tissue changes in liver are linked with histological abnormalities of kidney and gill. Once absorbed, the toxicant is transported by blood circulation to liver for transformation and /or storage, and if transformed in the liver it may be excreted through the bile or pass back into blood for possible excretion by kidney or gill (Lindstoma-Seppa *et al*., 1981). On account of this, the structure and functioning of the kidney are worth observing in any organism which undergoes physiological stress which will inevitably leave its effects on the vital organ. The following changes in kidney were observed in the present study in kidney on account of its exposure to low and higher sublethal concentrations of the pesticides, namely endosulfan and fenvalerate: fibrosis of kidney tubules, swollen glomeruli and their subsequent disintegration, extensive haemorrhage, hypertrophy of brush border in tubules and disorganization of tubules and enlarged periglomerular space. Most of the renal disorders due to toxicants mainly occur in kidney tubules but the glomeruli have also been affected in the present study. Similar effects have been observed by Dhanapackiam and Premalatha (1994) in *C. carpio* tested with malathion and sevin; Padgaonkar and Parab (1994) in *Etroplus maculatus* after exposure to DDT and Sulthana and Rajan (2007) in *O. mossambicus* treated with heavy metals. Gupta and Dalela (1987) have reported histological changes in the kidney of *Notopterus notopterus* exhibiting degeneration and dissolution of epithelial cells of renal tubules, hypertrophy and necrosis following subtle exposure to phenolic compounds. Similar observations have been made by Csepai (1978) in *C. carpio* chronically exposed to Anthio 40 EC, Satox and Basuden 10G, the organochlorine and organophosphate compounds. Murugesan (1988) has also observed the occurrence of fibrosis of kidney tubules in *H. fossilis* exposed to higher concentrations of textile mill effluent.

Ponniah and Kapoor (1998) have noticed histopathological changes in the kidney of *C. punctatus* exposed to chronic malathion toxicity such as glomerular shrinkage resulting in increased Bowman's spaces and vacuolation of epithelial cells lining proximal convoluted tubules and fibrosis of the renal tubules.

In the present study, *P. conchonius* exposed to sublethal concentrations of fenval, the fibrosis of glomerular tissues and accumulation of yellow pigments in them have been noticed as spotted by Murugesan (1988) in *H. fossilis* exposed to textile mill effluent and Madasamy (2001) in *A. lineatum* exposed to coconut husk retting effluent. This may be due to the effect of ingredients in the toxicant (Murugesan, 1988). Whereas, in endosulfan treated *P. conchonius,* the glomerular tissues have formed a thick "cyst-like" congregation. However, Das and Mukherjee (2000) have noticed alterations in the tubular cells rather than in the glomeruli of *L. rohita* exposed to hexacyclohexane and they have stated that it might be a defense mechanism in the fish to counter toxic metabolites.

Rita and Milton (2006) have observed various severe changes including degeneration and disorganization of glomeruli and vacuolation of epithelial cells of uriniferous tubules in *O. mossambicus* exposed to sublethal concentrations of cabaryl and cartap (carbamates) similar to the present study.

The kidney tissues of *P. conchonius* showed signs of damage due to exposure to sublethal concentrations of endosulfan and fenvalarate, as per all histopathological observations. Fish that experience severe physiological issues as a result of histopathological changes in their kidney tissue, as demonstrated in this study and other research, may eventually perish. In ummary, the results of these histological studies show a clear connection between pesticide exposure and the histopathological abnormalities seen in kidney tissues.

[**Abbrevations: bb-Brush border, bc-Bowman's capsule, bv-Blood vessel, d-Debris-cellular, dt-Distal tubule, ery-Erythrocyte, g-Glomerulus, l-Lumen, lt-Lymphoid tissue, mf-Muscle fibre, n-Nucleus, pct-Proximal convoluted tubule, p-Pigmented granules, rct-Renal collecting tubules]**

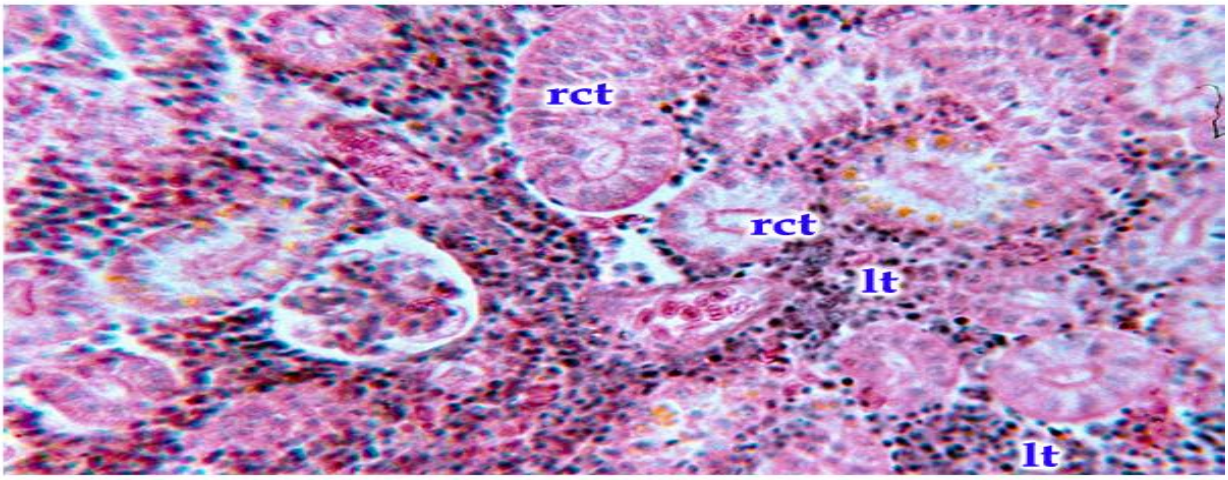
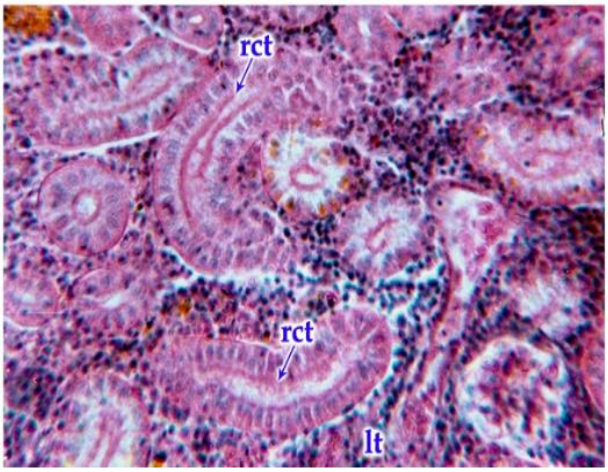


Plate 1

Plate No: 112 Shows T.S. and L.S. of primary and secondary renal collecting tubules, a normal glomerulus, minor blood vessels and capillaries and normal hemopoietic tissue.
Stain: Ehrlich's hematoxylin and Eosin **(Mag. 200x)**



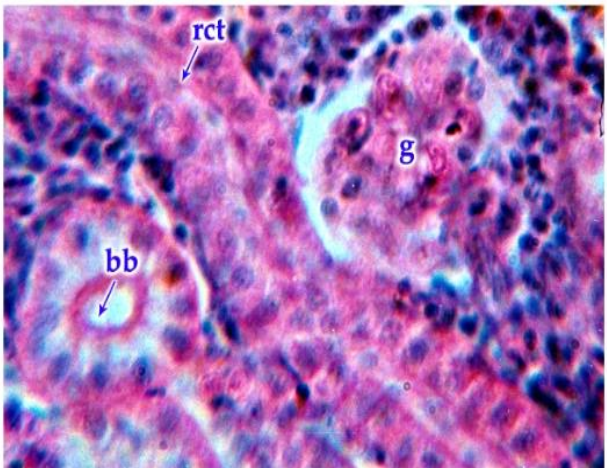


Plate 2 **Plate 3**

Plate No: 2 Shows T.S. and L.S. of renal collecting tubules, blood capillaries and normal hemopoietic tissue. Renal collecting tubules show normal brush borders.
Stain: Ehrlich's hematoxylin and Eosin**(Mag. 200x)**
Plate No: 3 T.S. and L.S. of renal collecting tubules and a glomerulus under high magnification: Renal collecting tubules show normal brush borders.
Stain: Ehrlich's hematoxylin and Eosin **(Mag. 200x)**

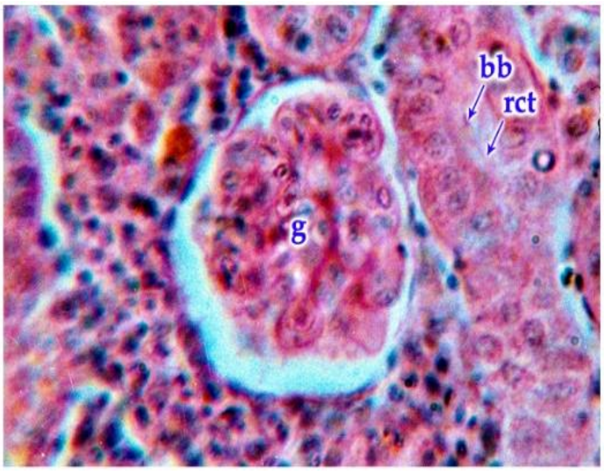


Plate 4

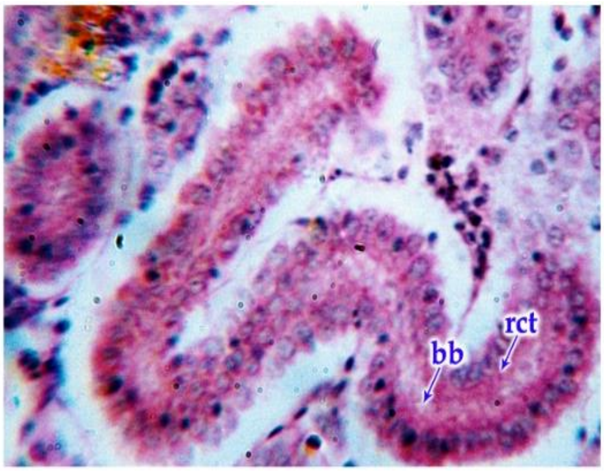


Plate 5

Plate No: 4 L.S. of a renal collecting tubule and a normal glomerulus, under high magnification.
Stain: Ehrlich's hematoxylin and Eosin **(Mag. 200x)**

Plate No: 5 Section of kidney under high magnification showing hypertrophied brush borders in primary renal collecting tubules in longitudinal section.
Stain: Ehrlich's hematoxylin and Eosin **(Mag. 200x)**

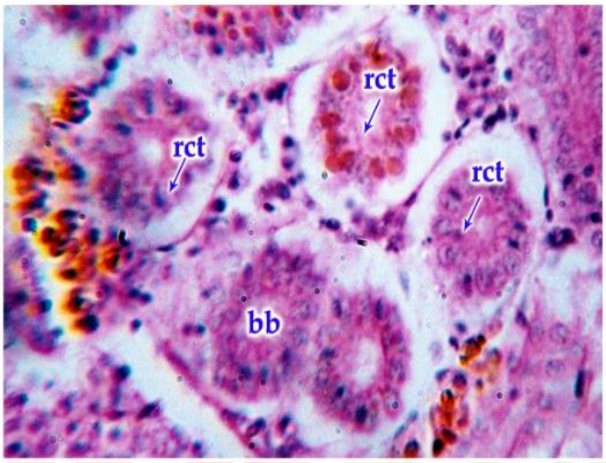


Plate 6

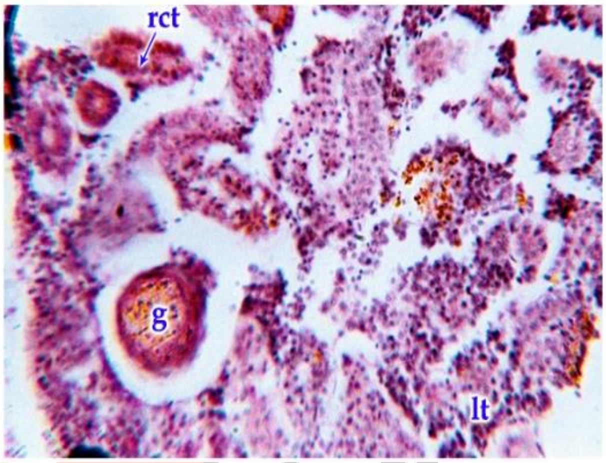


Plate 7

Plate No: 6 Section of kidney under high magnification showing hypertrophied brush borders in primary renal collecting tubules in transverse sections.
Stain: Ehrlich's hematoxylin and Eosin **(Mag. 200x)**

Plate No: 7 A section of Kidney under high magnification showing a completely disorganized glomerulus filled with yellow pigmented particles surrounded by connective tissue fibres. The surrounding area with dismantled renal collecting tubules.
Stain: Ehrlich's hematoxylin and Eosin **(Mag. 200x)**

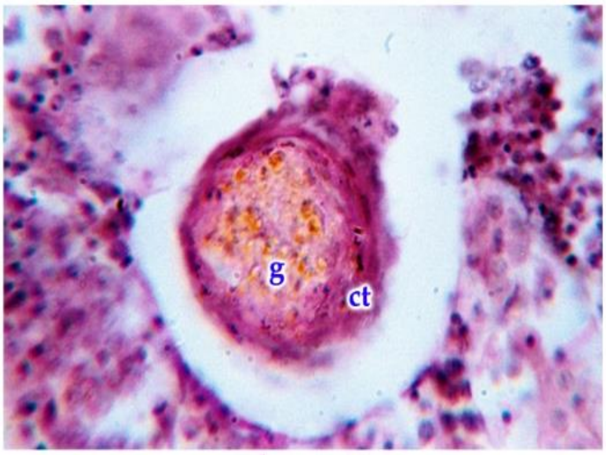


Plate 8

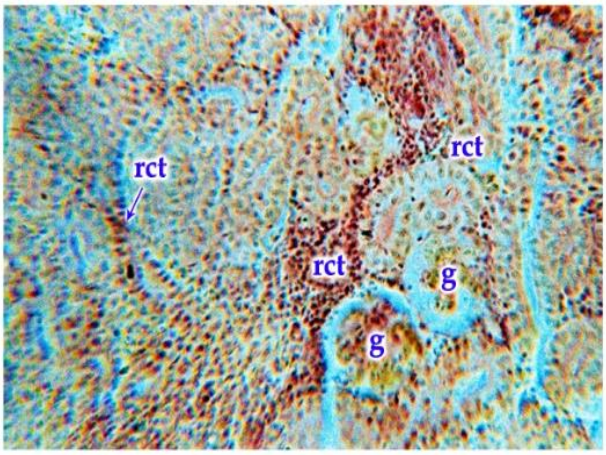
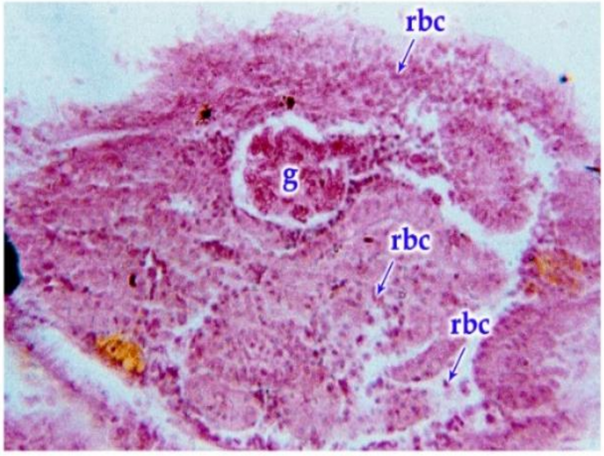


Plate 9

Plate No: 8 A portion of the previous plate under further higher magnification showing the disorganized glomerulus filled with yellow pigmented particles surrounded by connective tissue fibers.
Stain: Ehrlich's hematoxylin and Eosin **(Mag. 200x)**
Plate No: 9 Section of kidney showing a small glomerulus and stray R.B.Cs in entire section. The renal collecting tubules appear narrow with reduced lumen.
Stain: Von Gieson **(Mag. 200x)**



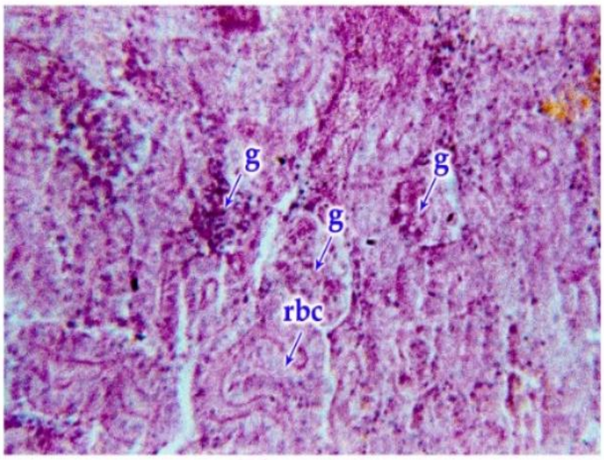


Plate 10 **Plate 11**

Plate No:10 Section of kidney showing a swollen glomerulus and stray R.B.Cs in entire section.
Stain: Ehrlich's hematoxylin and Eosin **(Mag. 200x)**
Plate No: 11 Section of kidney showing two glomeruli which are reduced in size and stray R.B.Cs in entire section. The renal collecting tubules also appear narrow with reduced lumen.
Stain: Ehrlich's hematoxylin and Eosin **(Mag. 200x)**

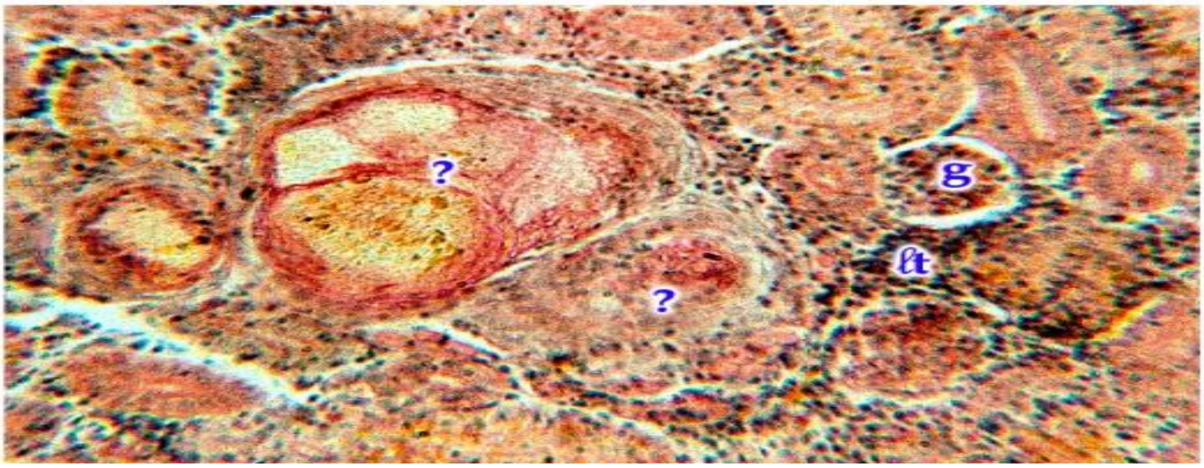
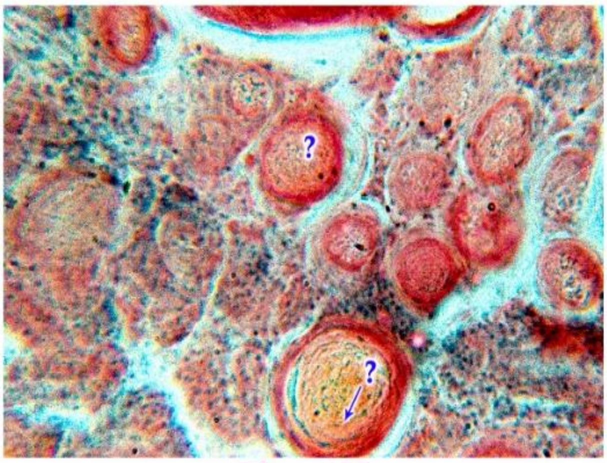


Plate 12

Plate No: 12 Normal glomeruli besides 'cyst' like congregations of tissues in to a body with a many layered 'cyst wall' surrounding a mass of disintegrating cells. It might be a cyst of a renal parasite or just a mass of tissue accumulation following disintegration of glomeruli owing to the deleterious effect of the chemical treatment.
Stain: Van Gieson **(Mag. 200x)**



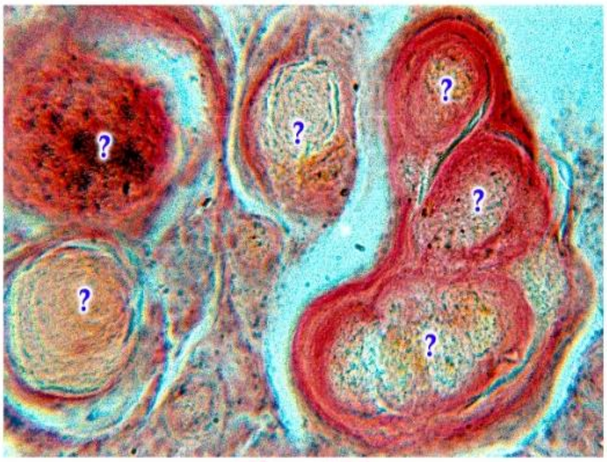
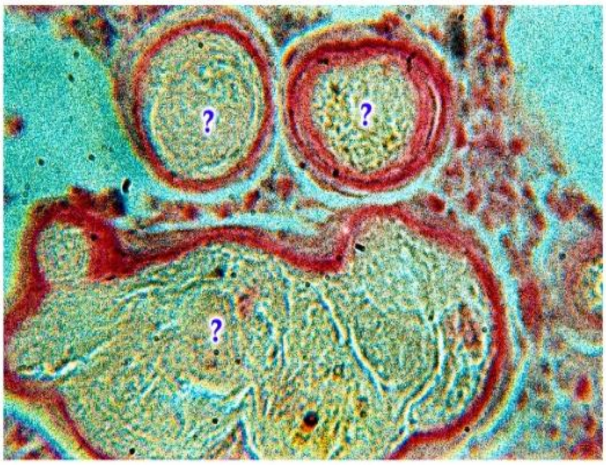


Plate 13 **Plate 14**

Plate No: 13 'Cyst' like congregations of tissues observed in the previous plate are found in the entire section in which the histology is completely lost.
Stain: Van Gieson **(Mag. 200x)**
Plate No: 14A highly aggravated condition in another part of kidney owing to the 'Cyst' like congregations of tissues are seen as observed previously.
Stain: Van Gieson **(Mag. 200x)**



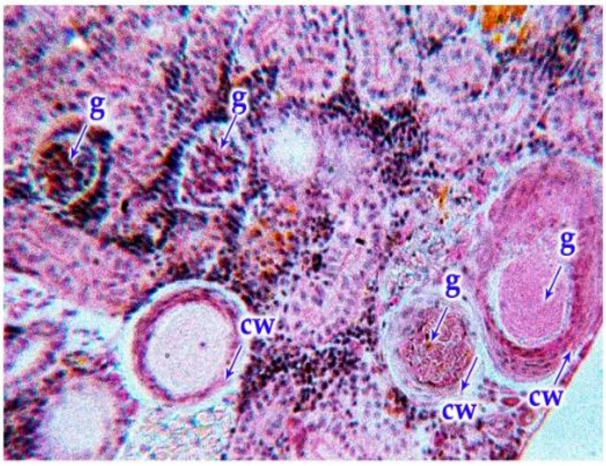


Plate 15 **Plate 16**

Plate No: 15 An extremely aggravated condition in another part of kidney owing to the 'Cyst' like congregations of tissues.
Stain: Van Gieson **(Mag. 200x)**
Plate No: 16 Normal glomeruli besides 'cyst' like congregations of tissues in to a body with a many layered 'cyst wall' surrounding a mass of disintegrating cells. It might be a cyst of a renal parasite or just a mass of tissue accumulation following disintegration of glomeruli owing to the deleterious effect of the chemical treatment.
Stain: Ehrlich's hematoxylin and Eosin **(Mag. 200x)**

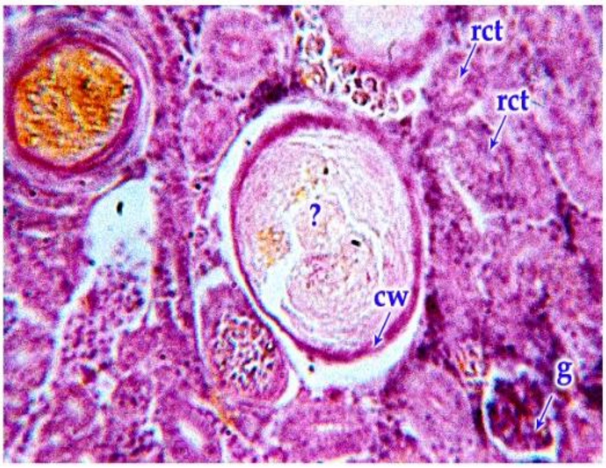


Plate 17

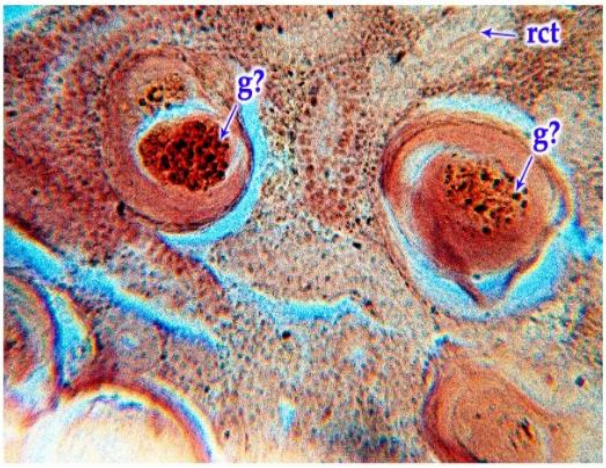


Plate 18

Plate No: 17 Normal glomeruli besides 'cyst' like congregations of tissues in to a body with a many layered 'cyst wall' surrounding a mass of disintegrating cells. It might be a cyst of a renal parasite or just a mass of tissue accumulation following disintegration of glomeruli owing to the deleterious effect of the chemical treatment.

Stain: Ehrlich's hematoxylin and Eosin **(Mag. 200x)**

Plate No: 18 Normal and disintegrated glomeruli are seen.

Stain: Von Gieson **(Mag. 200x)**

CONCLUSION

Both endosulfan and fenvalarate pesticides are highly toxic to the study animal even though their sublethal concentrations seem to be very low. Rosy barb is a common ornamental fish cultured by most of the aquarist and considered as a commercially important organism. Hence, agricultural spillage and run-off need to be closely monitored to conserve the fish species of human interest.

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