Journal Homepage: -www.journalijar.com
INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)
Article DOI:10.21474/IJAR01/9907
DOI URL: http://dx.doi.org/10.21474/IJAR01/9907
ISSN NO. 2320-5407

RESEARCH ARTICLE

HISTOPATHOLOGICAL EFFECTS OF GLYPHOSATE IN THE GILL, LIVER AND KIDNEY OF THE FRESH WATER FISH, CYPRINUS CARPIO.

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Manuscript Info

Manuscript History
Received: 14 August 2019
Final Accepted: 16 September 2019
Published: October 2019

Key words:-
Cyprinuscarpio, Glyphosate, LC_{50}, Histopathology.

Abstract

The common carp, *Cyprinus carpio* were exposed to $1/10^{th}$ of 96 hours LC_{50} concentration (0.02ppm) of glyphosate for 10, 20 and 30 days. Gill, liver and Kidney tissues of Fresh water fish, *Cyprinus carpio* were examined after exposure to sublethal concentration of Glyphosate**.** The gill showed histopathological changes like necrosis, vacuolar degeneration, fusion and atrophy of primary and secondary gill lamellae. Degeneration of cytoplasm in hepatocytes, atrophy, formation of vacuoles, rupture in blood vessels and disposition of hepatic cords were the histopathological changes noticed in the liver. The changes in the kidney include necrosis, cellular hypertrophy, granular cytoplasm and swelling of renal tubules.

Introduction:-

Water pollution is the contamination of water bodies usually as a result of human activities which is considered as one of the main problems of this century resulting from the addition of several contaminants in water systems by many ways and they change their natural qualities of water (Elezaby*et al*., 2001). Glyphosate, marketed by the trade name Roundup ®is a broad-spectrum, non-selective herbicide used for inhibition of unwanted weeds and grasses in agricultural, industrial urban, forestry and aquatic landscapes (Cavas and Konen, 2007). It is a highly water-soluble substance (10500 mg/l) with a half-life of about 3.5 to 90 days and in addition contains a cationic surfactant denominated polyoxyethylamine(POEA) that confers toxicological properties different from those of glyphosate(Folmar*et al*., 1979). Glyphosate-based herbicidesare known to be hazardous to the aquatic environment and are toxic to aquatic life with long lasting effects (Sihtmae*et al*., 2013, Tomlin, 2006).

Gills are considered the first target organ affected by contaminants in the water due to the persistent contact with the external environment (Perry and Laurent, 1993). The study of fish liver and kidney is also very important in the field of aquaculture induced by many problematic condition and aquatic pollution (Au, 2004). The examination of histopathology of various organs may therefore is a highly sensitive and accurate tool to assess the effects of xenobiotic compounds in field and experimental studies which reflecting the health of entire aquatic environment (Thophon *et al*., 2003). Hence, this study was undertaken to investigate the effects of different levels of Glyphosate in selected organs (gills, liver and kidney) of *Cyprinus carpio*.

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The fish, *Cyprinus carpio* measuring 6 to 8 cm length and 6.5 to 7.5 gm in weight irrespective of the sex were used in the experiment. Fishes were washed with 0.1 % $KMnO_4$ solution to avoid dermal infection. The precautions for maintaining the fishes were as per APHA, AWWA and WEF standards in the study. The fishes were exposed to herbicide, glyphosate to sublethal $1/10^{th}$ 96 hours LC_{50} value, i.e. 0.02 ppm concentration for 10, 20 and 30 days. When mortality occurred during the experimental period, dead fishes were removed immediately to avoid depletion of dissolved oxygen (DO) level which may adversely affects other fishes. The vital tissues like gill, liver and kidney of the fishes were taken out for histological study.

Microscopy Examination

At the end of exposure periods, 5 fish were taken from each replicate tank. The gill arches of the fish were excised from both sides. Fish were dissected, the abdominal cavity was operated and the liver and kidney were excised quickly and were fixed in Bouin's solution as a histological fixative for 24 hour (Tao, Liu, Dawson, Cao, & Li, 1999). According to Humason, (1967), the specimens were processed as usual in the recognized method of dehydration, cleared in xylene and finally embedded in paraffin wax before being sectioned at 5 µm using a rotary microtome (Leica RM 2235 Germany). The specimens were stained with hematoxylin and eosin. Finally, the prepared sections were examined and photographically enlarged using light microscopy (Hamilton compound photomicroscope).

Results and Discussion:-

Histopathologicl Lesions of Gills

In fishes, on each side of the buccal cavity four gills are present. The gill arch is composed of numerous gill filaments with two rows of secondary lamellae that run perpendicular to each filament. The composed cells in the secondary gill lamellae were contractile and separated the capillary channels. Within each capillary lumen, one to two erythrocytes were usually observed. At the base of lamellae, Chloride cells which were identified as large epithelial cells with light cytoplasm usually present. Histological study of the gills shows a typical structural organization of the lamellae in the untreated fish (Fig.1 A). The Glyphosate treated fishes showed several forms of histopathological changes such as cellular hypertrophy or hyperplasia in the epithelial layer of primary filaments and fusion of secondary lamellae (Fig. 1 B, C & D). During the experiment period, other changes occurred like epithelial lifting, interstitial edema and blood congestion in the vascular axis of primary filaments. In addition, a few telangiectasis were also observed in gill lamellae. The gills are important organs for respiration, osmoregulation, acid-base balance and nitrogenous waste excretion (Heath, 1987). They are directly exposed to poisons occurring in the external environment which often cause pathology in fish (Mallatt, 1985). The gills are among the most vulnerable structures of the teleost fish because of their external location and intimate contact with the water. So, they are liable to damage by any irritant materials whether dissolved or suspended in the water (Roberts, 1978). Our results suggest that there was a two-step process involved in the uptake glyphosate by fish gills: (1) adherence of the particles on the gill surface where mucus was attached; and (2) adsorption of glyphosate under conditions of the gill microenvironment. As the exposure period increases, the damages were severe in fish gills (Fig 1D).

Histopathologicl Lesions of Liver

The liver of control fish revealed the typical parenchymatous appearance. At the light microscopic level, the liver was divided into irregularly shaped lobules separated by the hepatopancreas and bile duct (Fig 2 A). The liver was made up of hepatocytes that were polygonal cells with a central spherical nucleus and a densely stained nucleolus.

After 10 days of exposure, the hepatocytes began to swell and slight infiltrations of leukocytes were observed (Fig 2 B). After 20 days period of exposure, the hepatocytes were still swelling. They showed extensive pyknosis and involution exhibiting darkly stained specks of necrotic nuclei as well as mild infiltration of leukocytes. There was some degeneration of the cell membrane and vacuolation in the cytoplasm (Fig 2 C). After 30 days, there were large vacuoles in the cytoplasm, the nuclei continued to be pyknotic and moderate infiltration of leukocytes was observed. There was a severe infiltration of leukocytes. The hepatocytes showed extensive pyknotic nuclei and large vacuoles in many areas (Fig 2 D).

According to Greenfield *et al*., (2008) histopathological biomarkers can be good indicators for the link between the action of the toxicant and the histological structure of the test organ. Ayoola (2008) found that the fish liver exposed to glyphosate had infiltration of leukocytes, increased hepatocyte size with pyknotic nuclei and presence of vacuoles. Ahmad *et al*., (2002) stated that disturbances in the osmotic regulation of cellular membranes resulted in increasing the volume of the nuclei and nucleoli and this lead to necrosis of liver cells. Similarly to Ayoola, (2008)

we consider that necrosis of some areas in the liver tissue were probably resulted from the excessive work required by the fish to get rid of the toxicant from its body during the process of detoxification by the liver.

Histopathologicl Lesions of Kidney

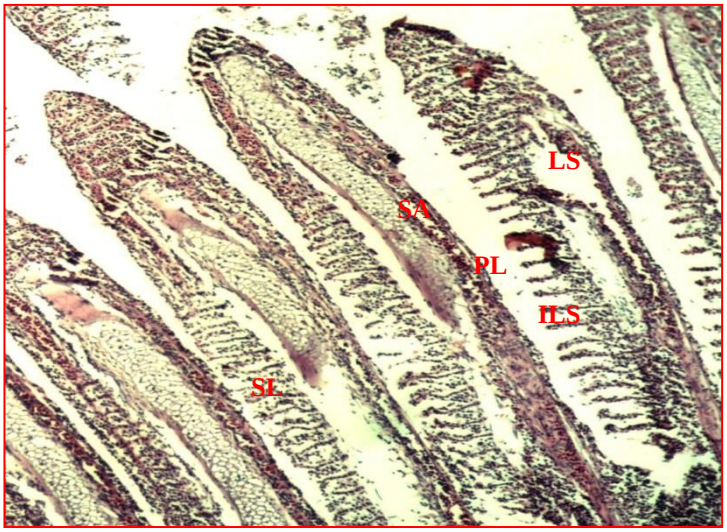
No recognizable changes were observed in the kidney of the control fish (Fig 3A). At the light microscopic level, the renal corpuscle was composed of the glomerulus and Bowman's capsule. The first proximal tubule was composed of cuboidal or low columnar cells with a well-developed brush border containing vacuoles and round basal nuclei.

After 10 days of exposure, the fish had renal lesions of varying degrees. Some glomeruli were collapsed or distorted. The epithelial cells of the first proximal tubule contained small vacuoles. Some of the cells were necrotic with small pyknotic nuclei (Fig 3B). After 20 days period of exposure, some of the proximal tubule epithelial cells still had small vacuoles. Some exhibited small hyaline droplets. After 30 days of exposure, they had extensive vacuoles and were swollen with pyknotic nuclei, the epithelium of many of the tubules had become exfoliated. The cytoplasm was pale and the cells appeared swollen. Pyknotic nuclei were observed in these tubules (Fig 3C). The present study also documented changes in inter renal cells and the presence of a large number of melanomacrophage centers (MMCs). The necrotic cells were removed by the MMCs, which showed defense mechanisms against the toxicants that entered the fish's body. Mela *et al.*, (2007) reported similar results in the fish, *Hoplias malabaricus* subjected to methyl mercury concentrations. They concluded that melanomacrophage centers are potent biomarkers of environmental degradation and pollution. Melanomacrophage centers are the deposition sites for materials of both endogenous (melanin, lipofuscin, hemosiderin) and exogenous (metals, biologically active particles) nature which were further suggested by Kurtovic *et al.*, (2008). Tetreault *et al.*, (2012) reported the enlargement of proximal tubules and a reduction of the Bowman's space which impaired the functioning of the kidney in fat head minnows collected from an effluent-dominated stream. According to Nsofor *et al.*, (2014), the hyaline droplets in the tubules were metal complexes excreted by the fish kidney, since normal kidney tubules function to modify the glomerular filtrate by reabsorption or secretion of inorganic ions.

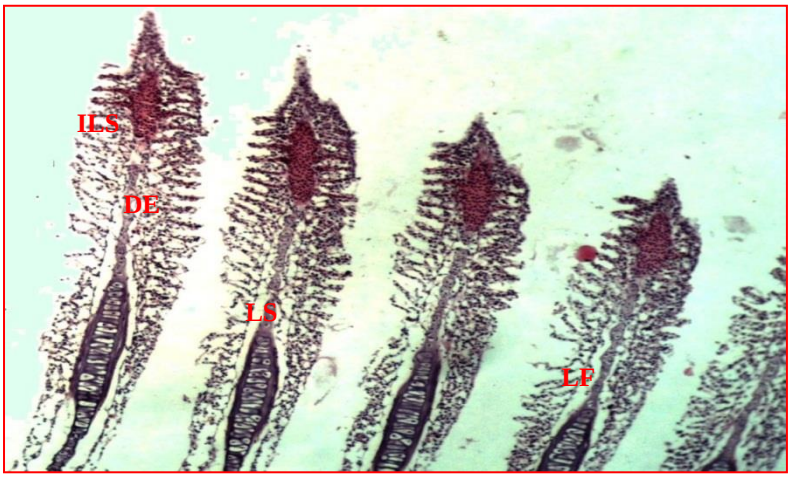
Conclusion:-

Our findings revealed that Glyphosate is toxic to fish and makes histological changes in fish organs. As the period of exposure increased, the fish organs like gill, liver and kidney showed severe damages compared with untreated fish. The fresh water fish, *Cyprinus carpio* are more susceptible to glyphosate; so its use on/near fish farm or in area close to aquatic environment should be discouraged.

Figure1a:-Control Gill Section of *Cyprinus carpio*

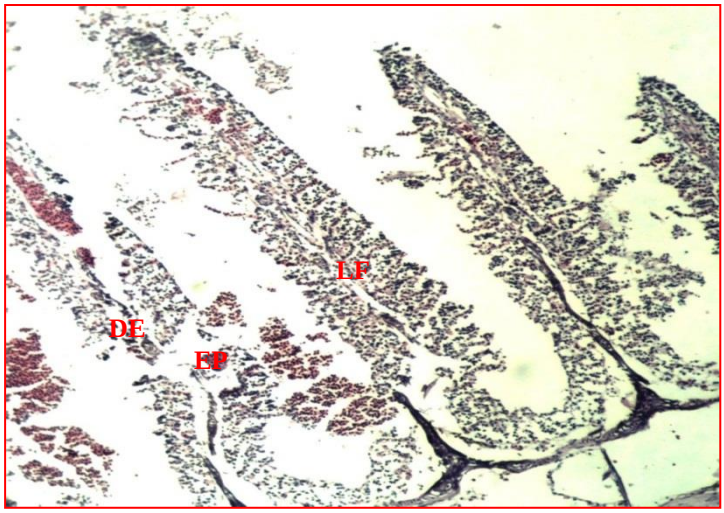


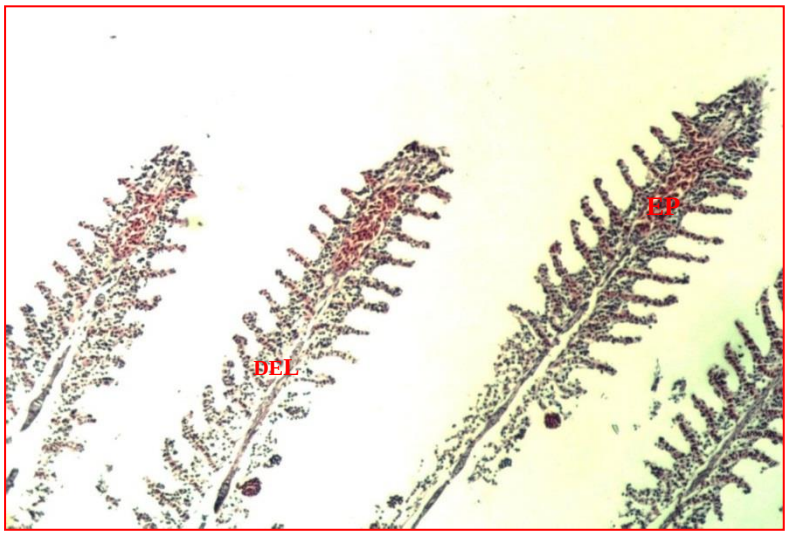
PL - Primary Lamellae
SL - Secondary Lamellae
LS - LamellarSpace
ILS - Inter LamellarSpace
SA - Supporting Axis



LF - Lamellar Filament
LS - Lamellar Space
DEL - Degeneration of Epithelial Lining
ILS - Inter LamellarSpace

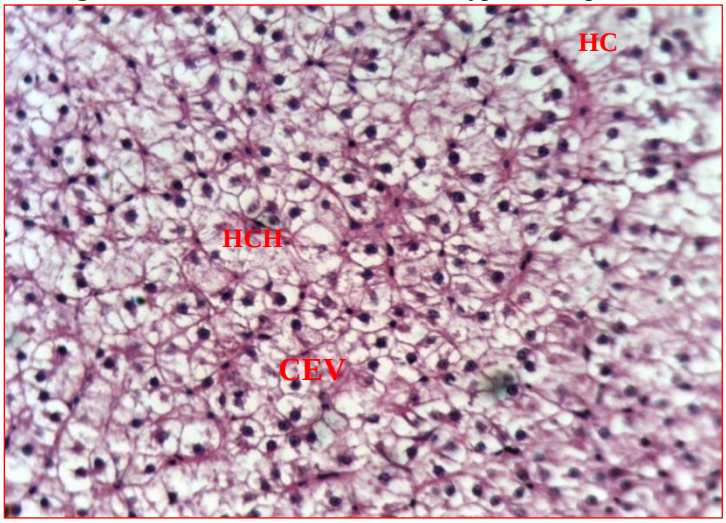
Figure 1c :-Gill section of fish exposed to 20 days of Glyphosate





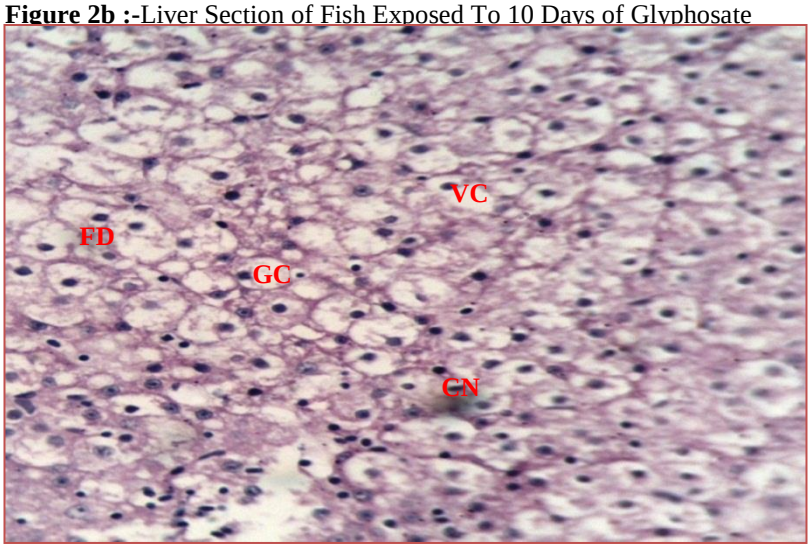
EP - Epithelial Proliferation
DEL - Degeneration of Epithelial Lining

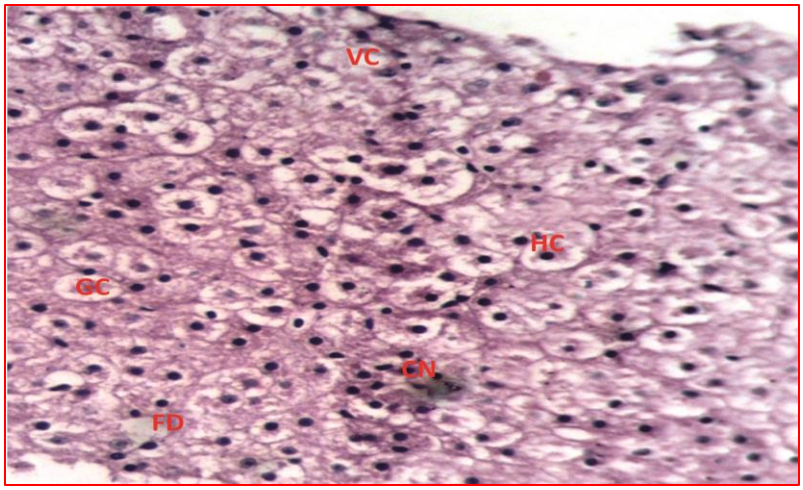
Figure 2a :-Control Liver Section of *Cyprinuscarpio*



HC - Hepatocyte Cells
HCH - Hepatic Cords
CEV - Central Efferent Vein

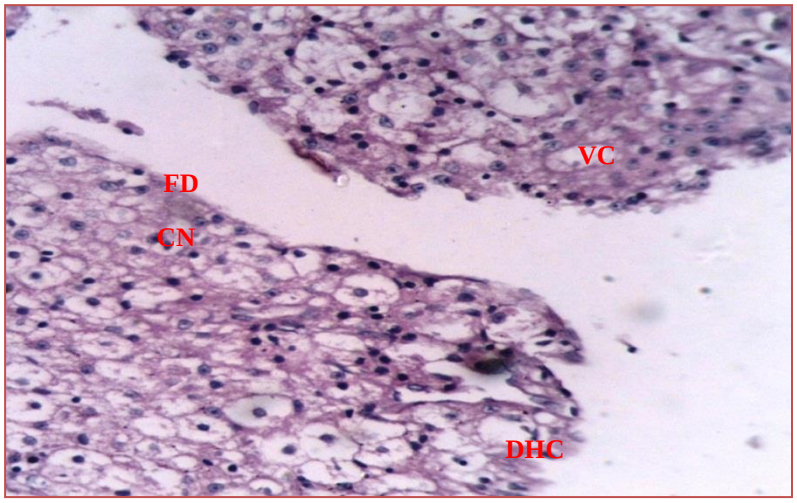
Figure 2b :-Liver Section of Fish Exposed To 10 Days of Glyphosate





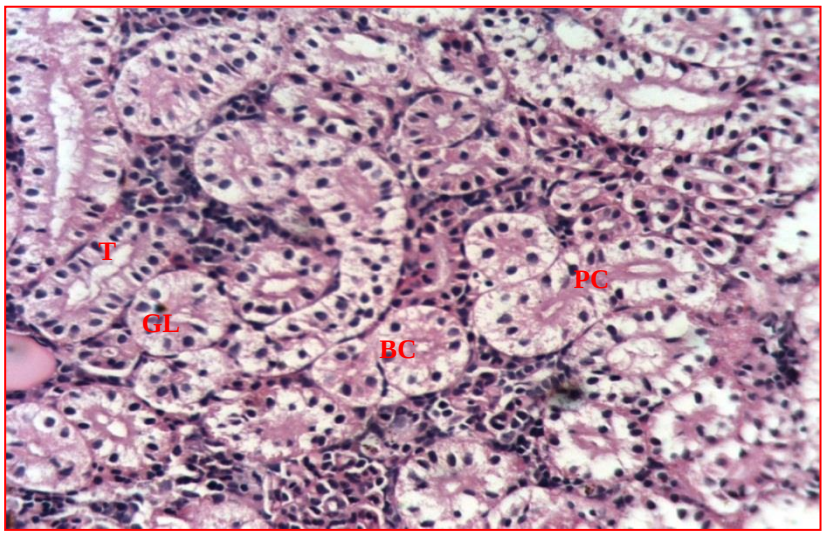
HC - Hepatocyte cells
VC - Vacuoles
GC - Gilssen's Capsule
FD - Fatty Degeneration
CN - Clumping of Nucleus

Figure 2d :-Liver Section of Fish Exposed To 30 Days of Glyphosate

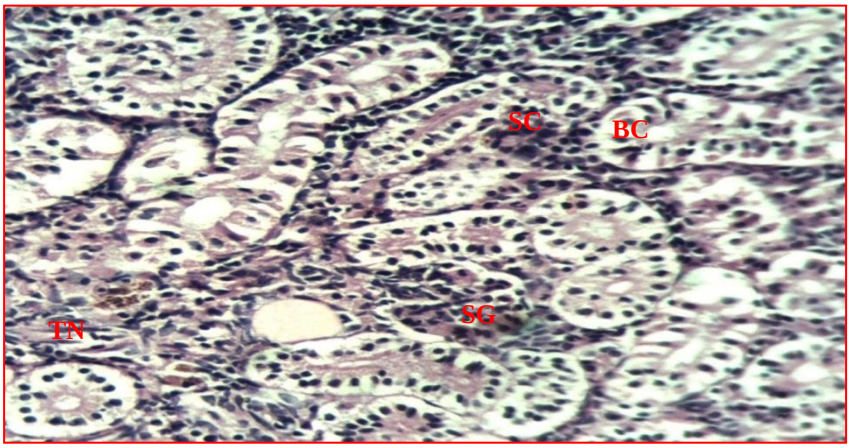


VC- Vacuoles, DHC- Degenerated Hepatocyte Cells, FD- Fatty Degeneration CN- Clumping of Nucleus

Figure 3A:-Control Kidney Section of *Cyprinus carpio*

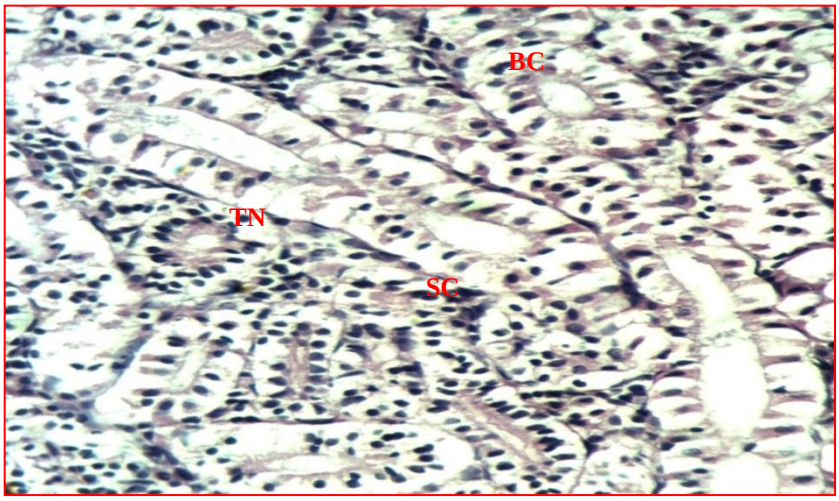


Kidney Section of Fish Exposed To 10 Days of Glyphosate



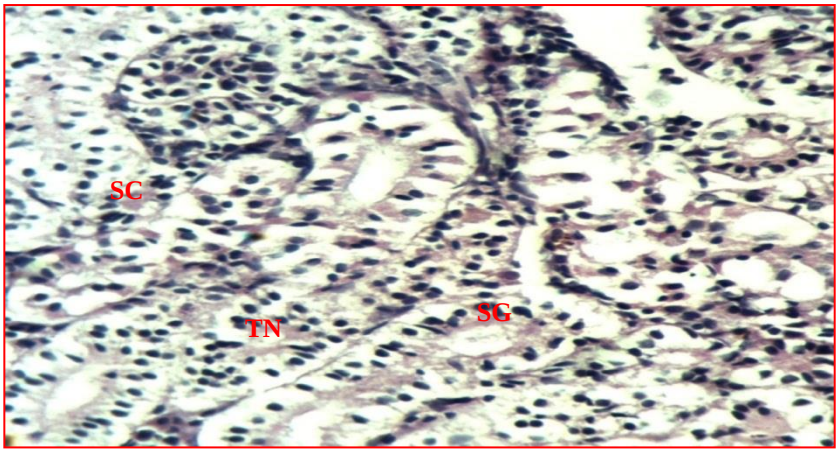
BC- Bowman's Capsule, SC- Shrunken of Cells, SG- ShrunkenedGlomerulus,TN- Tubule's Nucleus

Figure 3:-C Kidney Section of Fish Exposed To 20 Days of Glyphosate



BC - Bowman's Capsule
SC - Shrunken of Cells
TN - Tubule's Nucleus

Figure 3d:-Kidney Section of Fish Exposed To 30 Days of Glyphosate



SC- Shrunken of Cells, SG- Shrunkened Glomerulus, TN- Tubule's Nucleus

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