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HISTOPATHOLOGICAL CHANGES IN THE SELECTED TISSUES OF THE FRESHWATER FISH, *CIRRHINUS MRIGALA* AFTER EXPOSURE TO DICLOFENAC DRUG

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ABSTRACT
Fishes are appropriate as environmental toxicity bioindicator organisms both due to their role in the aquatic tropic chain and because of their sensitivity to low concentrations of toxic substances. The fingerlings were exposed to 2.8 per cent of sublethal concentration and experimental setup were made for short term exposure in 24, 48, 72 and 96 hours and long term exposure for 10, 20 and 30 days. Histopathological studies revealed severe tissue damage in gill, liver and kidney of the fish, *Cirrhinus mrigala* on exposure to short and long term exposure of Diclofenac drug.

KEYWORDS: Drug, *Cirrhinus mrigala*, acute, chronic.

INTRODUCTION

Pharmaceuticals are generally designed to have low toxicity but there is the potential for unintended side effects. These drug metabolites are polluting the water and air of this ecosystem, directly or indirectly. One of the most profitable industries of world are pharmaceuticals but unused medicines or excess usage leads to unstability of ecosystem leading to extreme harmful effects, whether it is death of birds or aquatic organisms.

Diclofenac is available as a generic drug in a number of formulations, including diclofenac diethylamine which is applied topically. It is used to treat pain, inflammatory disorders and dysmenorrhea. Due to its extensive use as analgesic and anti-rheumatic, diclofenac residues can nowadays be regularly detected in surface waters throughout the world.

Histopathological studies have been conducted to establish casual relationship between exposure and various biological responses (Schwaiger *et al*., 1996). They provide a better assessment of fish health, as well as the effects of pollution on each biochemical parameter. Histopathological changes have been integrated with the impact of various stressors such as microbial pathogens, toxic compounds, nutritional and adverse environmental conditions (Marchand *et al*., 2009).

One of the great 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, accumulation and biotransformation of xenobiotics in the fish (Gernhofer *et al*., 2001).

MATERIALS AND METHOD

Cirrhinus mrigala is the freshwater carp found in Northern India, Punjab, West Bengal and Orissa. It has a wider mouth and thinner lips. Body silvery, dark grey alomg with the back, sometimes with coppery tinge. Adult attains a maximum length of 90 – 100 cm and a weight of 1.4 to 2.8 kg. The growth of about 20 cm is recorded in 8 months. This growth data is based on an experiment conducted on 6000 fingerlings.

ANALYTICAL TEST FOR WATER CHEMISTRY

The tap water free from contaminants was used as dilution water for the present study. The physico – chemical analysis of water used in the experiments was carried out using the methods of APHA, (2005).

Physico – chemical parameters of the tap water used for the present study are as follows: Temperature 27.2 ± 0.9 (0C), pH 7.1 ± 0.1, Dissolved oxygen 5.4 ± 0.4 (mg/l), Calcium 120 ± 1.1 (mg/l), Magnesium 50 ± 0.2 (mg/l).

COLLECTION AND MAINTENANCE OF FISH

The fingerlings of the freshwater fish, *Cirrhinus mrigala* ranging in weight from 4 kg to 8 kg and measuring (4 cm to 6 cm in length) were procured from Aliyar. The procured bulk samples of *Cirrhinus mrigala* were transported to the laboratory in well aerated polythene bag and acclimatized to the laboratory conditions under natural photo period for one week in large plastic containers at (26 ± 5 ^{0}C). The tank was previously washed with potassium permanganate to prevent any fungal infection. The fishes were maintained in dechlorinated tap water of the quality used in the test and water was renewed every day to provide freshwater rich in oxygen.

Continuous artificial aeration was maintained throughout the acclimation and exposure periods. During the periods of acclimation they were fed everyday with oil cake mixed with rice flour. Unhealthy fish and those with infections were removed. Feeding was stopped two days prior to the experiment to maintain same state of metabolic requirements. Fish belonging to both sexes were selected for the present investigation. All the precautions laid down on recommendations of the toxicity tests to aquatic organisms are followed Anon, (1975).

TOXICANT

Analytical grade Diclofenac 2 [(2,6 – Dichlorophenyl) amino] benzene acetic acid sodium salt [DCF, 99.9% pure]; CAS No.15307-79-6 were purchased from Sigma-Aldrich chemic GmbH, Germany, Dimethyl sulphoxide (DMSO) (CAS No 67-68-5) was purchased from Fischer Scientific India Pvt. Ltd, India and 0.2 ml/l used to prepare the stock solution at different concentrations (1, 10 and 100 µg/L due to their low water solubility. IUPAC Name is 2 [(2, 6 – Dichlorophenyl) amino] benzene acetic acid sodium salt. Molecular formula is $C_{14}H_{10}C_{12}NNaO_2$ and molecular weight is 381.13.

EVALUATION OF MEDIAN LETHAL CONCENTRATION

The concentrations of the pollutant at which 50 percent of the test animals die during a specific test period of time is referred to as median lethal concentration (LC_{50}) or median tolerant limit. In aquatic toxicology the traditional LC_{50} test is often used to measure the potential risk of a chemicals (Jack de Bruijin *et al*., 1991).

Batches of 10 healthy fishes were exposed to different concentrations of drug, Diclofenac to calculate the LC_{50} value. One more set of fishes are maintained as control in tap water. To find the wide range of concentration 100-600 ml were chosen and the number of dead or affected fishes was counted at regular intervals upto 48 hrs. The level of the dissolved oxygen, pH, alkalinity and hardness were monitored and maintained constant.

Appropriate narrow range of concentration was used to find the median lethal concentration, using a minimum of 6 fishes for each concentration and the mortality was recorded for every 24 hrs upto 96 hrs. It was found as for 96 hrs, using probit analysis method (Finney, 1971). From the stock solution various sublethal concentrations were prepared for bioassay studies.

Three groups of fishes were exposed to 1/10 of the drug 'Diclofenac' for 24, 48, 72, 96 hrs, 10 days, 20 days and 30 days respectively. Another group was maintained as control. All the groups received the same type of food and other conditions were maintained similarly. At the end of each exposure period, fishes were sacrificed and tissues such as gill, liver and kidney were dissected and removed. The tissues (10 mg) were homogenized in 80% methanol, centrifuged at 3500 rpm for 15 minutes and the clear supernatant was used for the analysis of different parameters.

HISTOPATHOLOGICAL STUDY

Freshwater fish, *Labeo rohita* were exposed to 24 hrs, 48 hrs, 72 hrs, 96 hrs, 10 days, 20 days and 30 days to a sublethal concentration of Diclofenac drug. At the end of exposure period, fish were randomly selected for histopathological examination.

Tissues like gills, liver and kidney were isolated from control and experimental fish. Physiological saline solution (0.85% Nacl) was used to rinse and clean the tissues. They were fixed in aqueous Bouin's solution for 48 hrs, processed through graded series of alcohols cleared in xylene and embedded in paraffin wax. Gills alone were processed by double embedding technique. Sections were cut at 6µ thickness stained with Haemotoxylin Eosin, dissolved in 70% alcohol (Humason, 1962) and were mounted in Canada Balsam. The photographs at 200x magnification were taken with computer aided microscope (Intel play Q x 3, Intel Corporation, Made in China).

RESULTS AND DISCUSSION

The exposure of the fresh water fish, *Cirrhinus mrigala* to 2.8 per cent of Diclofenac drug. These drug for short and long term duration (24, 48, 72 and 96 hours and 10, 20 and 30 days) lead to the formation of histopathological lesions of varying intensities on the gill tissues and internal organs like liver and kidney.

Gill histology of the control fish revealed the intact nature of both primary and secondary gill lamellae. The secondary lamellar surface was covered with simple squamous epithelial cells and capillaries separated by mucous cells. Each primary gill lamellae was flat leaf like structure. It consisted of double rows of secondary lamellae with the central supporting axis. They were situated laterally on either side of the interbranchial septum.

When the fish was exposed to short term exposures like 24, 48, 72 and 96 hours the gill showed degeneration of epithelial lining, fusion of secondary lamellae with

irregular lamellar spaces and necrosis were observed. In long term exposure for 10, 20 and 30 days congestion with infiltration by the chronic inflammatory cellular exudates, collapsed secondary lamellae were noted.

Liver consisted of hepatic cells and connective tissues called lattice fiber which supported the hepatic cells. Hepatocytes were located among the sinusoids and they formed cord like structures the hepatic cell cords. In short term exposure the liver showed symptoms of general necrosis, degeneration of hepatocytes, clumping of nucleus, fatty degeneration and cloudy swelling. The long term exposure showed karyolysis, rupture in blood vessels and portal triads were infiltered with chronic inflammatory cells.

Body kidney composed of many nephrons and interstitial lymphoid tissues. The glomerular capsule was formed of an inner and outer layer of single flattened epithelia. Renal tubules consisted of a single layer of epithelial cells. When the fish was exposed to short term exposure of Diclofenac drug, the kidney showed degenerative changes with dilated glomeruli and Bowman's capsules, severe necrosis, shrunkened glomerulus and nephritic changes. In long term exposure the kidney showed intercellular spaces, tubular necrosis, cloudy swelling of renal tubules and pycnotic nuclei.

Miron *et al*. (2008) reported edema and fusion of the secondary lamellae in gills of silver catfish exposed to ammonia levels above 96 hour LC_{50}. They also suggested that all these lesions may reduce gill functional surface for gaseous exchange, impairing respiratory function. Alterations in epithelial lifting, hyperplasia and hypertrophy of the epithelial cells, besides partial fusion of some secondary lamellae are examples of defence mechanisms.

Girish and Bijoy, (2014) have reported the liver cells were degenerated and the normal architecture of liver was markedly disorganized. Congestion of central vein, clumped erythrocytes and haemorrhages were observed in the liver of exposed fishes. Saroch *et al*. (2014) observed the histological observation exhibited progressive degenerative changes which are marked by shrinkage of glomeruli, increase in Bowman's space, damage of haematopoietic tissue and necrosis in tubular cells in *Heteropneustes fossilis* (bloch) by the exposure of cadmium chloride. Yancheva *et al*. (2016)) observed the histological changes in different fishes.

HISTOPATHOLOGY OF THE GILL OF *Cirrhinus mrigala*

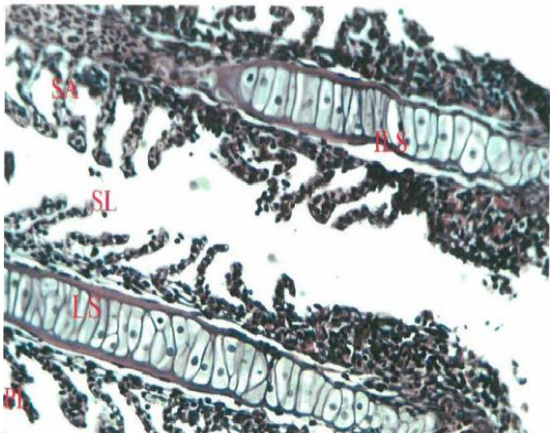

Fig: 1. Control gill section

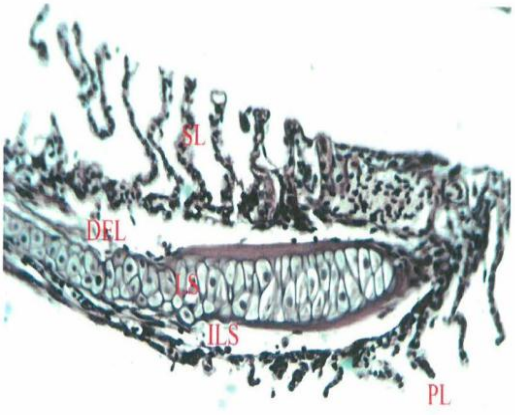

Fig 2. Gill section of 24 hours

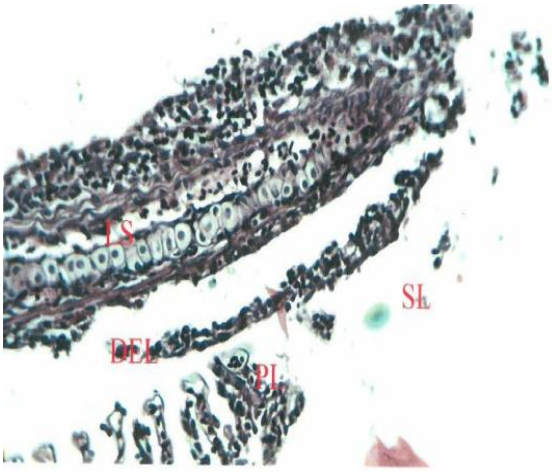

Fig: 3. Gill section of 48 hours

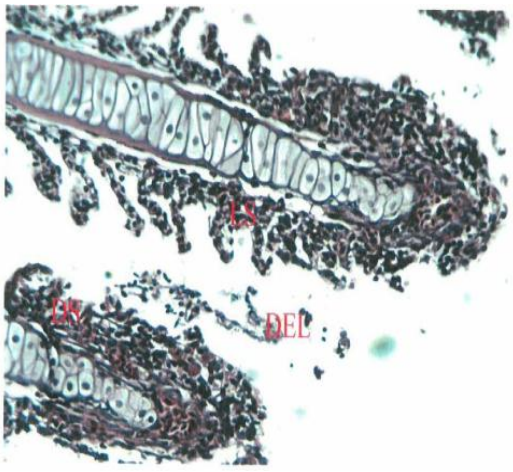

Fig: 4. Gill section of 72 hours

PL – Primary lamellae
SL- Secondary lamellae
LS- Lamellar space
ILS- Inter lamellar space
SA- Supporting axis
DEL-Degeneration of epithelial lining

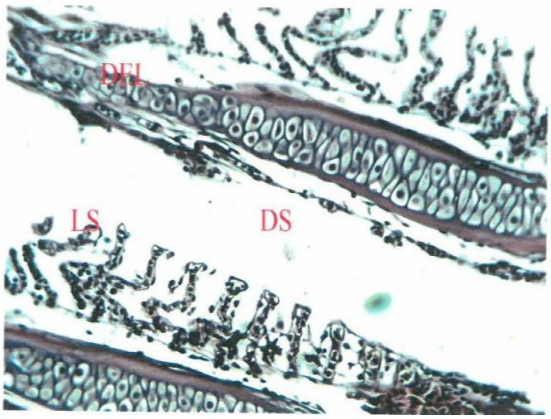

Fig: 5. Gill section of 96 hours

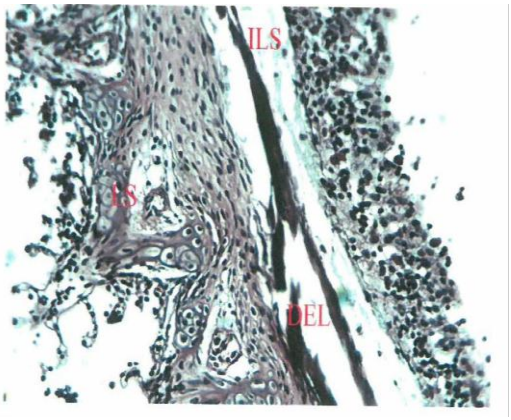

Fig: 6. Gill section of 10 days

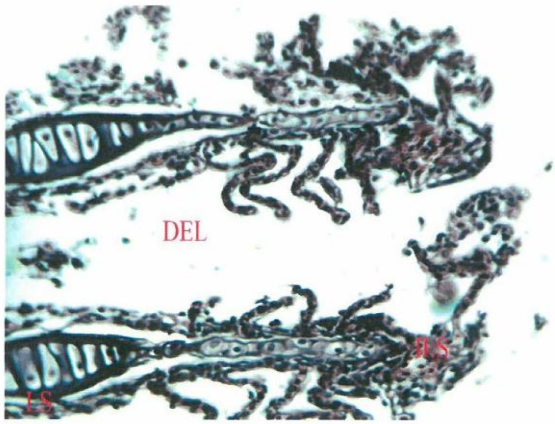

Fig: 7. Gill section of 20 days

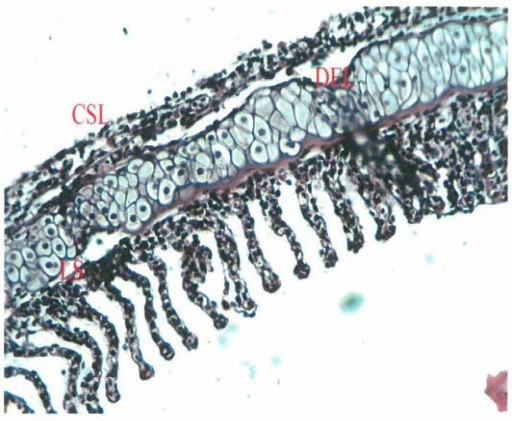

Fig: 8. Gill section of 30 days

DS- Degenerated secondary lamellae
LS – Lamellar space
ILS- Inter lamellar space
DEL- Degeneration of epithelial lining
CSL- Collapsed secondary lamellae

HISTOPATHOLOGY OF LIVER OF *Cirrhinus mrigala*

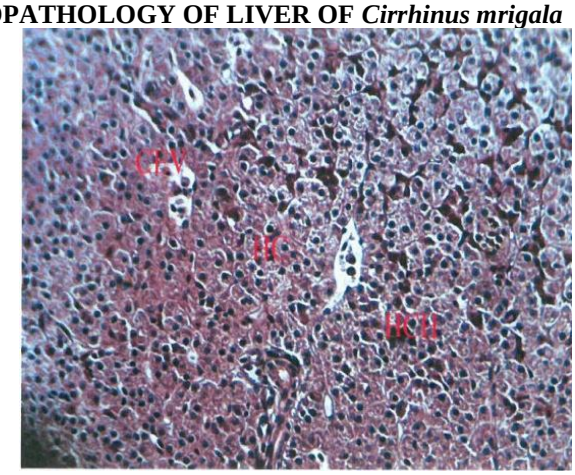

Fig: 9. Control Liver section

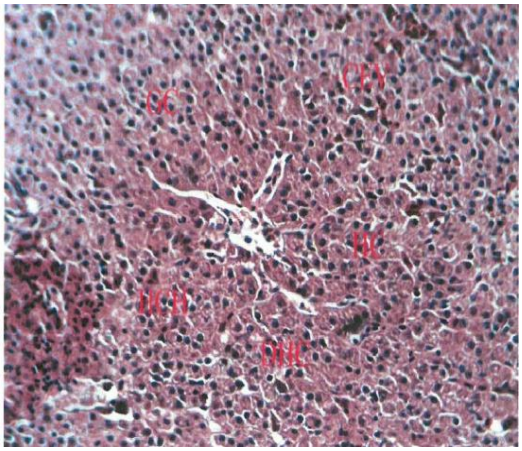

Fig: 10. Liver section of 24 hours

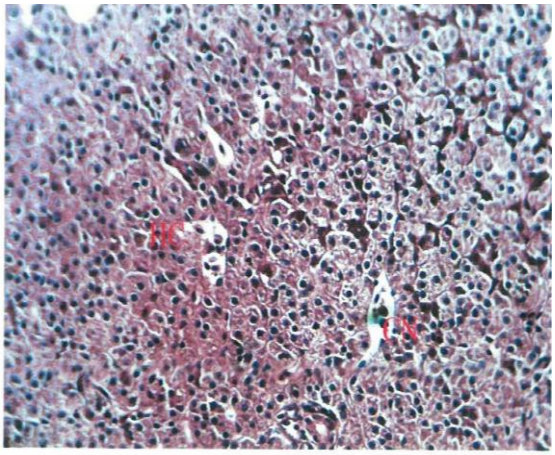

Fig: 11. Liver section of 48 hours

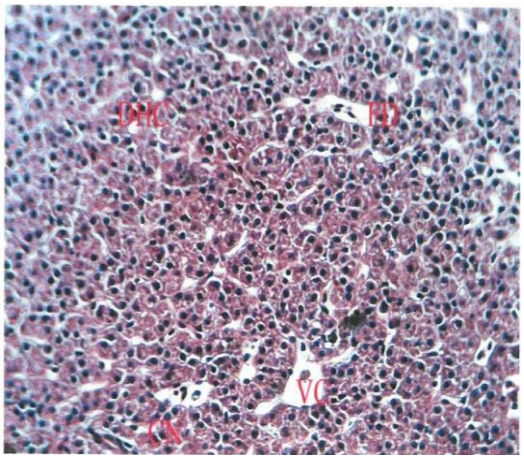

Fig: 12. Liver section of 72 hours

HC- Hepatocyte cells
HCH- Hepatic cords
CEV- Central efferent vein
GC- Gilssen's capsule
DHC- Degenerated hepatocyte cells

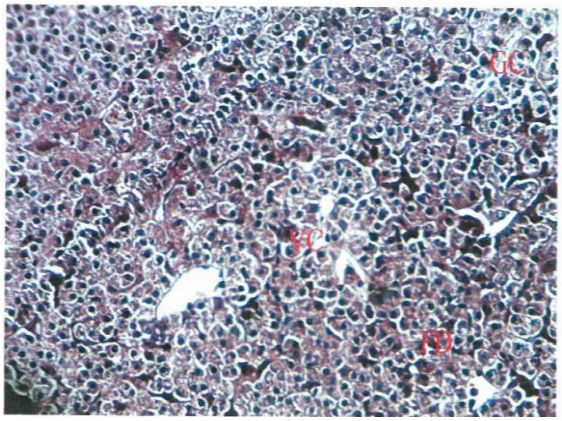

Fig: 13. Liver section of 96 hours

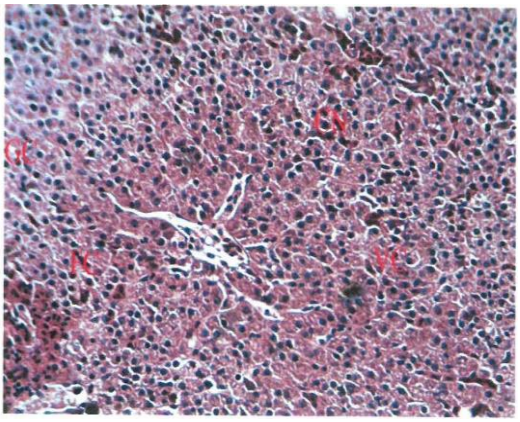

Fig: 14. Liver section of 10 days

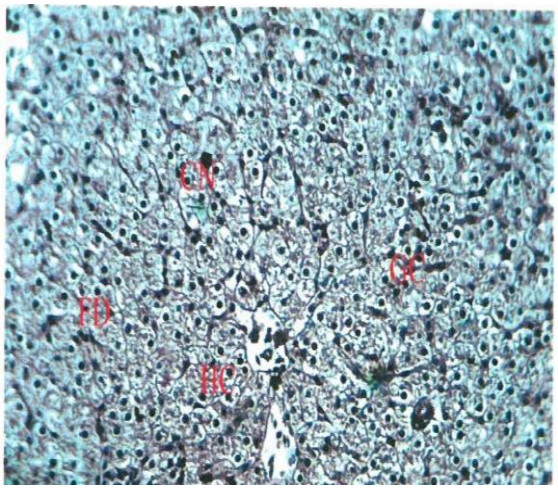

Fig: 15. Liver section of 20 days

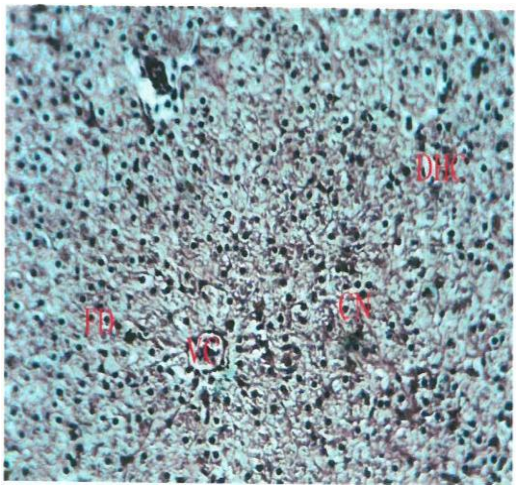

Fig: 16. Liver section of 30 days

GC- Gilssen's capsule
FD- Fatty degeneration
VC-Vacuoles
CN-Clumping of nucleus
HC-Hepatocyte cells
DHC- Degenerated hepatocyte cells

HISTOPATHOLOGY OF KIDNEY OF *Cirrhinus mrigala*

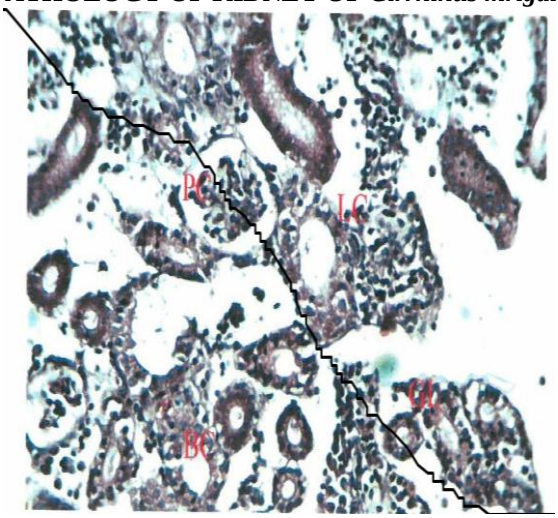

Fig: 17. Control Kidney section

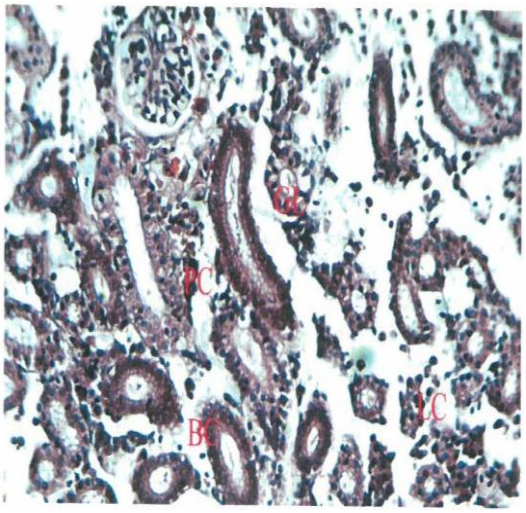

Fig: 18. Kidney section of 24 hours

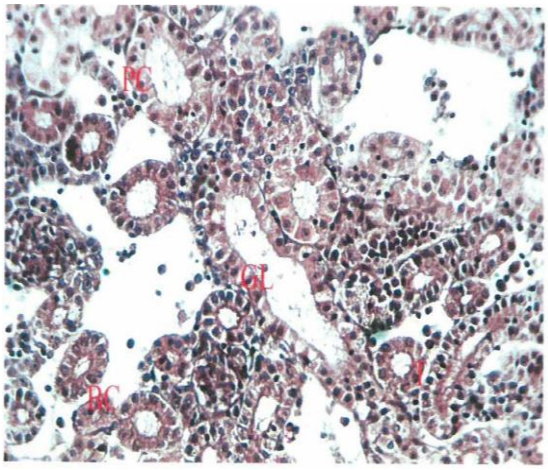

Fig: 19. Kidney section of 48 hours

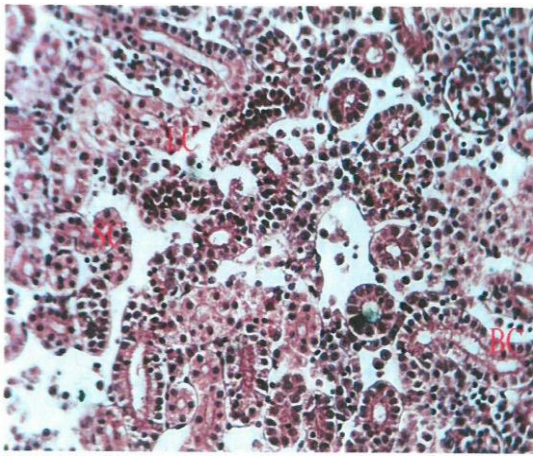

Fig: 20. Kidney section of 72 hours

GL- Glomeruli
LC- Lymphoid cells
PC- Parenchyma cells
BC- Bowman's capsule
T- Tubules
SC-Shrunkened cells

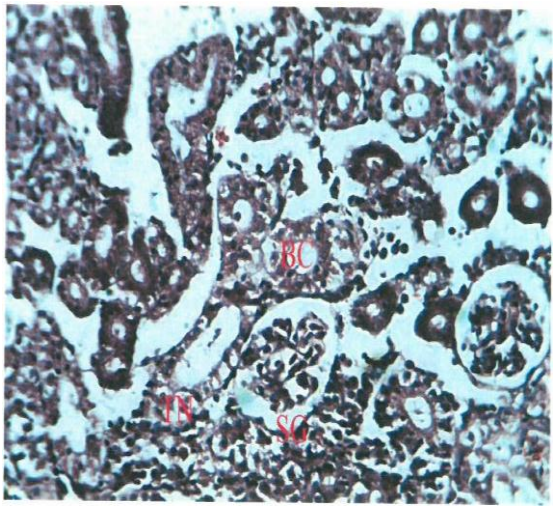

Fig: 21. Kidney section of 96 hours

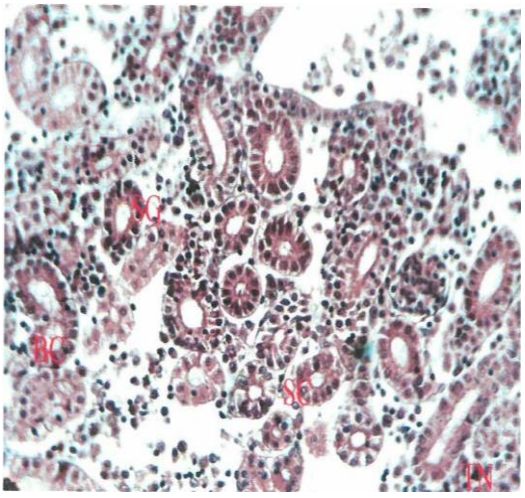

Fig: 22. Kidney section of 10 days

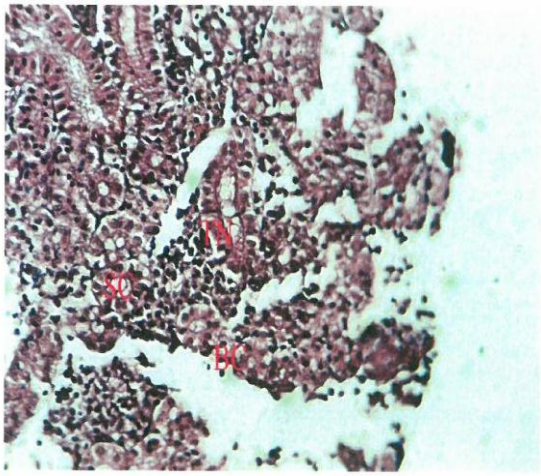

Fig: 23. Kidney section of 20 days

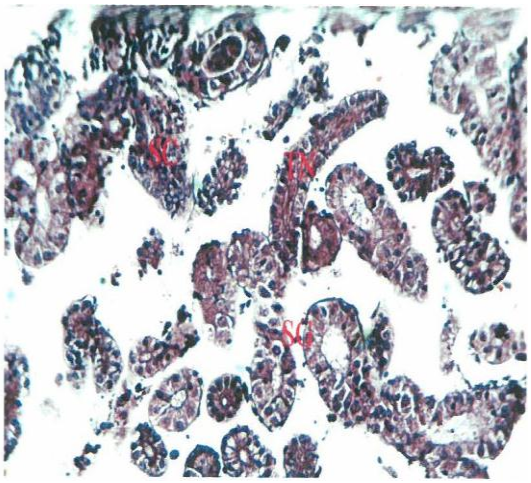

Fig: 24. Kidney section of 30 days

BC- Bowman's capsule
SG- Shrunkened glomerulus
TN- Tubules nucleus
SC- Shrunkened cells

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