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Histopathological Changes Induced by Sub Lethal Toxicity of Nuvan in the Liver and Kidney of Fresh Water Fish *Channapunctatus.* (Bloch.)

Suneel Kumar
Department of Zoology, School of lifesciences, Khandari Campus,
Dr. B.R.A. University, Agra, Utter Pradesh, India
R. K. Gautam
Department of Zoology, School of lifesciences, Khandari Campus,
Dr. B.R.A. University, Agra, Utter Pradesh, India

Abstract:
There are many different types of environmental pollutants which are able to kill a large number of animals, birds and even human beings with-in few seconds. The dangerous effects of pollution in our environment on account of large scale use of pesticides. Nuvan (Dichlorovas) is a member of chemicals organophosphorus compounds. It is extremely toxic to insects and is widely used as insecticides. It is affective against flies, aphids, spider, mites, caterpillars, thirps and white flies in green house, outdoor fruits and vegetables and crops. Nuvan has been taken for the acute toxicity to a fresh water fish Channa punctatus LC_{50} value calculated for the acute toxicity, which were 0.024 ml/L. Histopathological study of kidney and liver after 7^{th}, 14^{th}, 21 and 28 days exposure to sub lethal toxicity of Nuvan. The aim of this study is to show the alteration in histopathological changes in liver and kidney and get the real picture of toxicological effect of organophosphate pesticide Nuvan,and correlated to know about the adverse consequence of environmental toxicant on human health. The study is very important from histopathological point of view.

Keywords*: Nuvan, Channa punctatus, Histopathology, Toxicity, Liver, Kidney*

1. Introduction

Unprecedented growth in human population has posed a serious problem to fulfill the requirement of sufficient amount of food to every citizen of the country. The use of crop -protecting toxicants and pesticides now become a necessity of farmers. But when chemicals, fertilizers and pesticides, applied on the field, its effect on survival, growth, metabolism and reproduction on non targeted organisms. Pesticides are biologically active chemicals of great value to agriculture. Pesticides applied directly to the soil are carried away by rains and floods as runoff to the water bodies and this alters the physico-chemical properties of water .Exposure of organisms to xenobiotics such pesticides, is a serious matter in environmental and toxicological chemistry.

Among synthetic pesticides organophosphates are widely used in agriculture, health and hygiene programs due to their high effectiveness as insecticide but less persistence in the environment. The use of organophosphates started after the detection of their inhibitory effects on acetylcholinesterase (Koelle&Gilman 1949) 1.Nuvan(DDVP) is a systemic organophosphate insecticide used widely for controlling insect pest of fruits, vegetables and crop plants. Like other organophosphates, nuvan is also an acetylcholinesterase inhibitor and primarily works as nerve poison. This is very toxic insecticide and has been classified as possible carcinogen by USEPA based on occurrences of tumors in mice and rated as moderately hazardous by W.H.O.

The fish is a good indicator and highly sensitive in such ecosystem where the water gets contaminated to toxic chemicals. Specific lesions occurring in organs of fish exposed to toxic substances under laboratory conditions are helpful as biomarker of exposure. As a result histopathological examination is increasingly being recognized as a valuable tool for assessing the impact of environmental pollutants on fishes (Tehet al., 1997; Handyet al., 2002)2,3. Sub-lethal concentrations are usually considered safe because they do not cause death. But, as the liver and kidney serves many vital functions and a major route of excretion of metabolites of xenobiotics, and receives the largest proportion of postbranchial blood, and therefore, it is more likely to undergo histopathological alterations under pesticide stress (Ortiz et al., 2003)4.

The present work is an effort to assess the toxic impact of Nuvan on the histology of kidney and liver tissues of common carp Channa punctatus. This is a fast growing food fish of very high economic importance and easily breeds in confined waters.

2. Materials and Methods

Clinically healthy fresh water fish Channa punctatus were collected from the local fish market of Agra, district (U.P.).The average length and weight of fish is 12-15 cm and 60-70 g respectively. They were kept in glass aquarium (75 x 37.5 x 37.5) capacity 25 liter, having non chlorinated tap water aquaria bath 1% $KMnO_4$ solution for disinfection. The fish were acclimatized for one week before examination. The water used for toxicity test contained 20-25^oC and 7.2 pH during acclimations, and they were feed readymade market fish food twice in a day .Feeding was stopped 24 hr before starting the experiment. Dead fish (If any) was

removed from aquaria as soon as possible to avoid water fouling and water was changed after 2 or 3 days.Organophosphate Pesticide, Nuvan (DDVP) from Syngenta India Ltd. was use for present study. Five aquaria were set up for each concentration and each aquarium contained six fish in 25 L dechlorinated water. The data was analyzed statistically by log dose /probit regression line method (Finney, 1971)5, which was calculated 0.024ml/L lethal dose applied for 24h,48h, 72h and 96hour toxicity test. One control group of healthy fish was maintained simultaneously. Water quality monitoring was done prior to the experiment, during the experiment and after the experiment. Fish Channa punctatus were sacrificed at 7, 14, 21 and28day of exposure.Fish were first immobilized in ice and then dissected out carefully;Liver and kidney were removed and fixed in bouins fluid for 24 hr and then processed and embedded in paraffin for block preparation. The section were cut at 5 micron and stained in hematoxylin and eosin. Prepared slides were examined under light microscope and photographed for histopathological effects.

3. Results and Discussion

The microscopically histopathological observations in the liver of fish Cnannapunctatusafter exposed to sub lethal concentration of nuvan,noticed several histological alterations in compared to the control (Fig.-1). After7days of nuvan exposure in fishes were predominantly shown in the hepatocytes were radially from central vein at place. There were increases sinusoidal spaces also cirrhosis, mild necrosis and fat accumulation. Some of the hepatocytes are accumulate cytoplasmic granules and shrinkage leading to damage of the cytoplasmic material in the liver cells (Fig.-2).After 14 days exposure of nuvan toxicity, liver shown pathogenesis with many lesion. Most important histopathological changes were the necrosis and inflammation in the sinusoidal tissue. The hepatocytes were ischemic condition i.e. the lack of blood in the tissues (Fig.-3).While after 21 days exposure toxicity showed remarkable changes i.e. cloudy swelling and extension of sinusoids,fibrosis and cirrhosis in hepatic lobules and nuclear necrosis. The hepatocytes show hepatic vacuolization (fig.-4).The most remarkable changes in the liver were observed after 28 days of nuvan toxicity. The architecture hepatocytes loss of polygonal shape of hepatocytes, degeneration, focal necrosis and loss of cell boundaries of giant cell were also develop (Fig.-5).Similar findings have been supported by Mathur (1962, 1965, and 1976) in Ophiocephaluspunctatus, Barbus stigma, Trichogasterfasciatuas and Heteropneustesfossilis due to DDT, dieldrin and lindane toxicity. Konar(1970),Chakraborty, Amminikutty and Rege (1977),Anees (1978),Dubale and Shah (1979) and Awasthi (1984) reported hypertrophy of hepatic cells, loss of characteristic polygonal shape of liver cells, degeneration, shrinkage, rupture swelling necrosis margination of cells vacuolation, centralobular, perilobular hypertrophy, loss of cell boundaries resulting in to binuclear hepatocytes. Formation of gaint cells, splitting of the tissue, disintegration of hepatic cords, neoplasia and atrophy due to various pesticides exposure in fresh water fishes. Shastry and Sharma (1979) observed hypertrophy of hepatic cells and liver corrdisary, vocuolatuion of cytoplasm and necrosis, rupture of hepatic cells membrane and necrotic centro lobular area in fresh water fish Channa punctatus due to a sub lethal concentration of aldrin. Mandal and Kulshrestha (1980),Qureshiet al. (1983),Kulshrestha and Jauhar (1984),Bhatnagaret. al. (1987) and Ramalingam (1988) reported cytoplasmic degeneration of nuclei in liver tissues and vacuolation in hepatic cells and ruptured of blood vessels due to organophosphate pesticides in fresh water fishes. Desai et al. (1984) have been reported necrosis and vacuolization of hepatocytes and fat degeneraton from the tissues due to the organophosphate monocrotophas toxicity exposure in the liver of Tilapia mossambica.Mathur (1965) observed the vacuolation and necrosis in the liver ofOphiocephaluspunctatus due to pesticidal toxicity. Elezabyet al. (2001) studied hemorrhage, necrosis and lipidosis in the liver OreochromisniloticusandClariasgariepinusdue to malathion and organophosphorus insecticide (Hostathion) toxicity. Similar histopathological changes were reported in the fresh water fish Anabas testudinesexposed to paper mill effluents (Nanda and Panigrahi, 2004). King (1962) observed several histopathological changes in liver of guppies and brown trout after DDT exposure. Jordnoska and Kostoski (2005)reported several histopathological changes due to pesticides toxicity in fresh water fish Barbusmeridionalispetenyi(Heckal.)

The histopathological observations have been observed in the kidney after 7 days of nuvan toxicity shows cloudy swellings on renal tubules, several variations in size and cellularity in glomeruli to normal. The tubules were showing focal necrosis at various places (Fig.7). After 14 days several hyper cellular glomeruli were seen with much vascular degeneration of the tubular cells and displacement of nucleus in renal cells. The renal tubules were shown mild necrosis of interstitial haematopoetic tissue and hyperchromatic nuclei and widening of the renal tubular lumen (Fig. 8). While after 21 days of nuvan toxicity kidney shows pathogenesis with many lesions. Several hypertrophy in glomeruli and loss of haemopoeitic tissue and lack of blood supply in tissue and nephrosis(Fig.9).An interesting and remarkable changes in kidney after 28 days compare to control, shows chronic inflammation of interstitial tissue, internal hemorrhage. The scattered area of fibrinoid necrosis as well as ischemic brinkling of glomeruli were observed (Fig.10).

These findings similar to Mathur (1962),Konar(1970),Shastry and Sharma (1979),Mandal and Kulshrestha (1980),Kulshresthaet al. (1984),Radhiahet al. (1986),Saxena (1988) and Bhatnagaret al. (1989) have been reported the rupture and flattening of the cells of renal epithelium, displacement of nuclei in renal cells, widening of the renal tubule lumen, shrinkage of glomerulus, haemorrhage of blood vessels and clumping of erytherocytes and carbaryl pesticides in fresh water fishes. Dubale and Shah (1981) and Dhanapakiam and Premlatha (1994) reported the consequent necrosis of cell and vacuole formation in Cyprinuscarpio due to malathion and sevinvacuolation, necrosis, loss of nuclei, ruptured glomerulli, cellular debris and accumulation of dark granules in fresh water fish Channa punctatus due to phenyl mercuric acetate (PMA) toxicity.Annes (1976)and Pandey (1996) have observed rupture and flattering of renal epithelium cells, displacement of nuclei in renal cells, widening of the renal tubular lumen, eosinophilic costs in the tubular lumina, rupture of renal peritonium, shrinkage of glomerullus, haemorrhage of blood vessels, necrosis of interstitalhaematopoetic tissue and hyperchromatic nuclei in the fresh water fish due to pesticide toxicity. Das and Mukherjee, (2000) reported dilation of renal tubules and necrotic changes characterized by karyorrhexix and karyolysis in Labeorohita exposed to hexachloro-cyclohexane.Tilaket.al.(2001) noticed severe necrosis,cloudy swelling in the renal tubules, cellular hypertrophy,granular cytoplasm and vacuolization in kidney tissues of Ctenopharyngodonidella after exposure to fenvalerate.Degeneration in the epithelial cells of renal tubules,pycnotic nuclei in the hematopietictissue,dilation of glomerular

caplillaries,degeneration of glomerulus,intra cytoplasmic vacuoles in the epithelial cells with tubular lumen were observed in the kidney tissues of fish exposed to diltamethrin(Cengiz,2006).Valmuruganet.al. (2007) reported pycnotic nuclei in tubular epithelium, hypertrophied epithelial cells of renal tubules, contraction of glomerulus and expension of space inside the Bowmans capsule in the kidney of Cirrhinusmrigala exposed to monocrotophos.

4. References

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Appendix

Explanation of figures:- Liver Photo figure. (1-5), Kidney photo figure. (6-10)

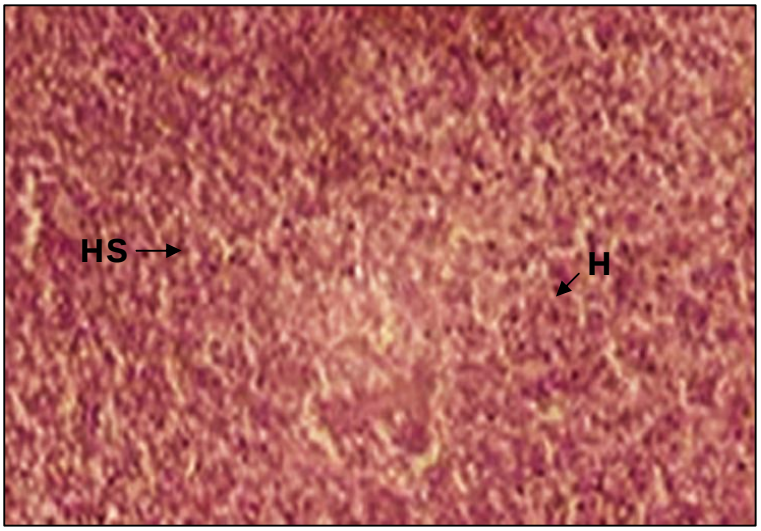

Figure 1: Liver (Control)

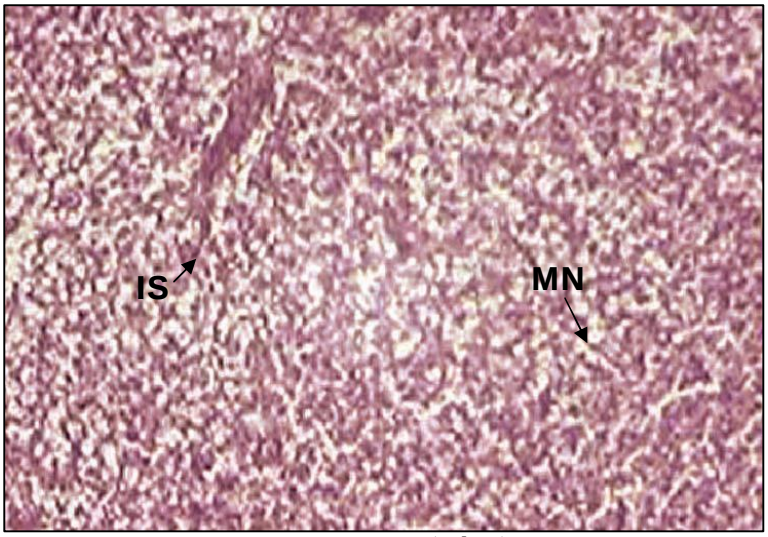

Figure 2: Liver (7 days)

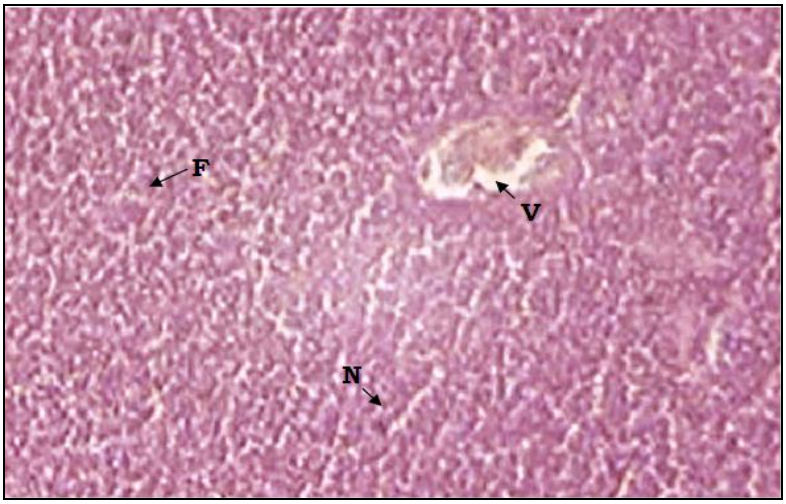

Figure 3: Liver (14 days)

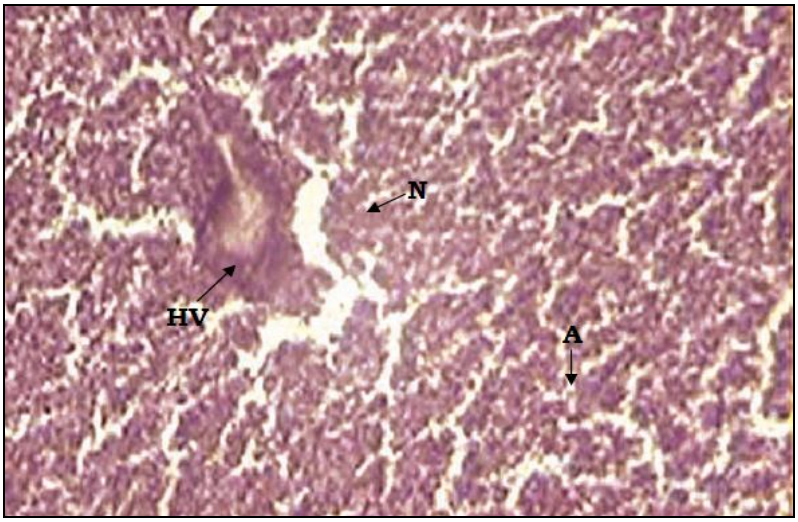

Figure 4: Liver (21 days)

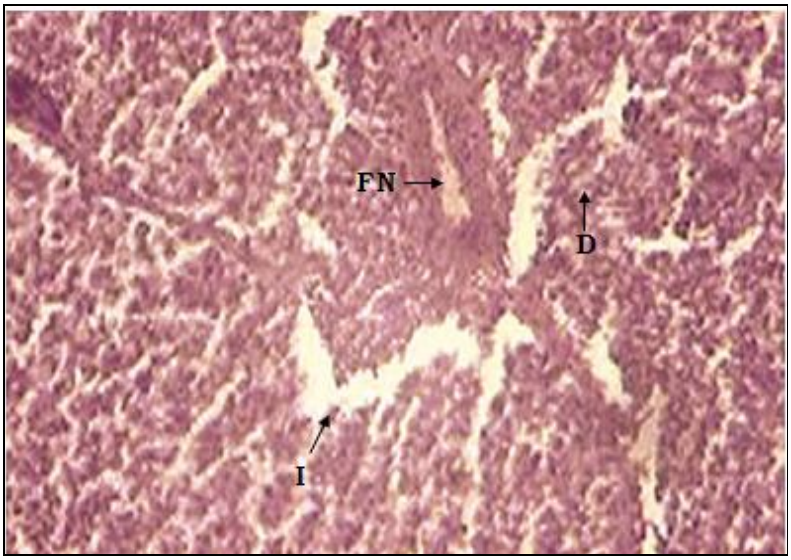

Figure 5: Liver (28 days)

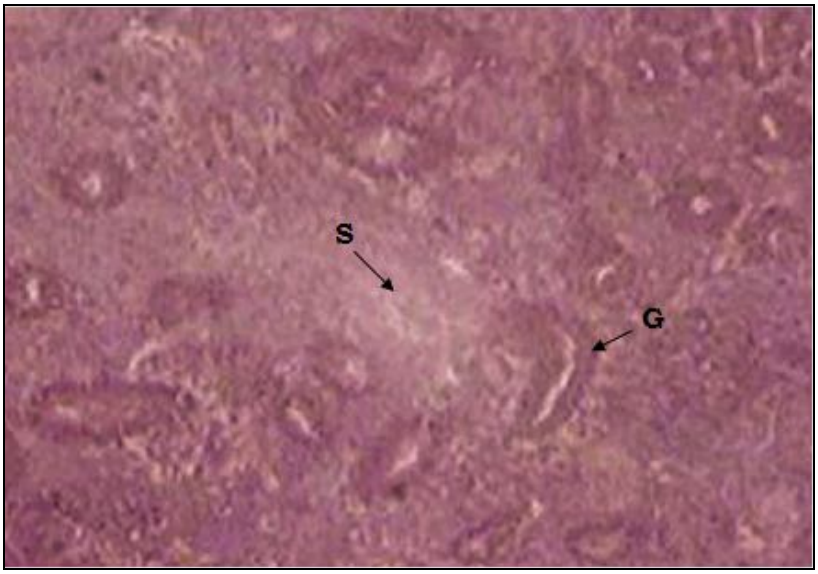

Figure 6: Kidney (Control)

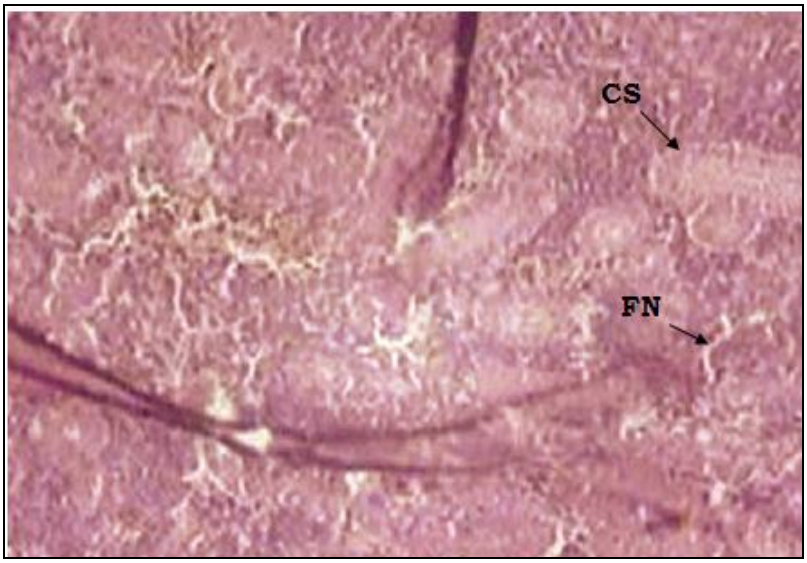

Figure 7: Kidney (7 days)

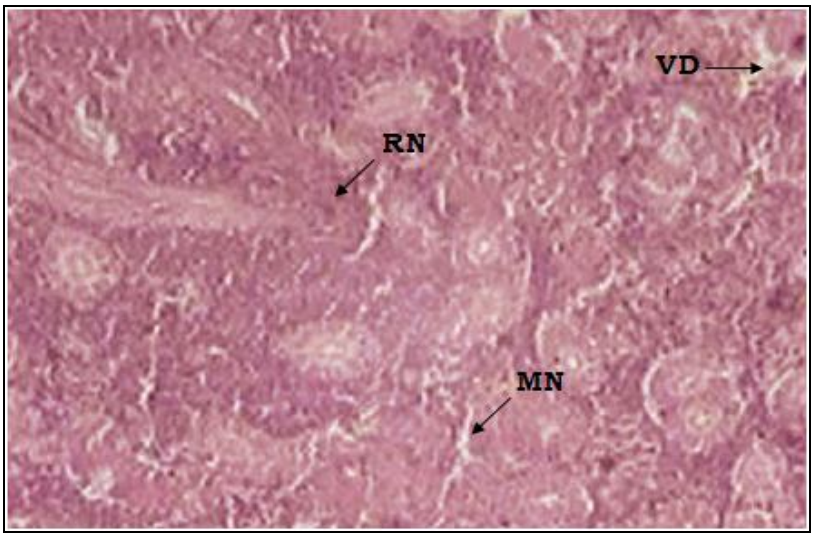

Figure 8: Kidney (14 days)

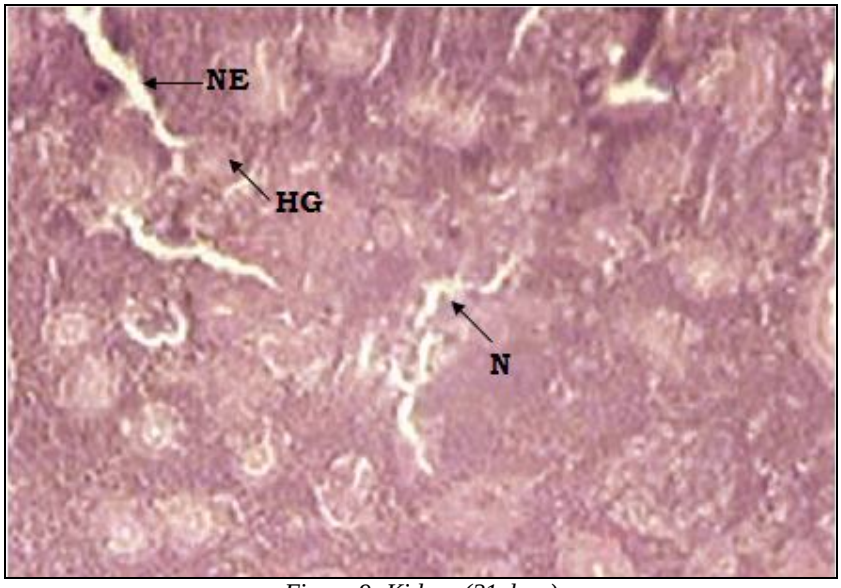

Figure 9: Kidney (21 days)

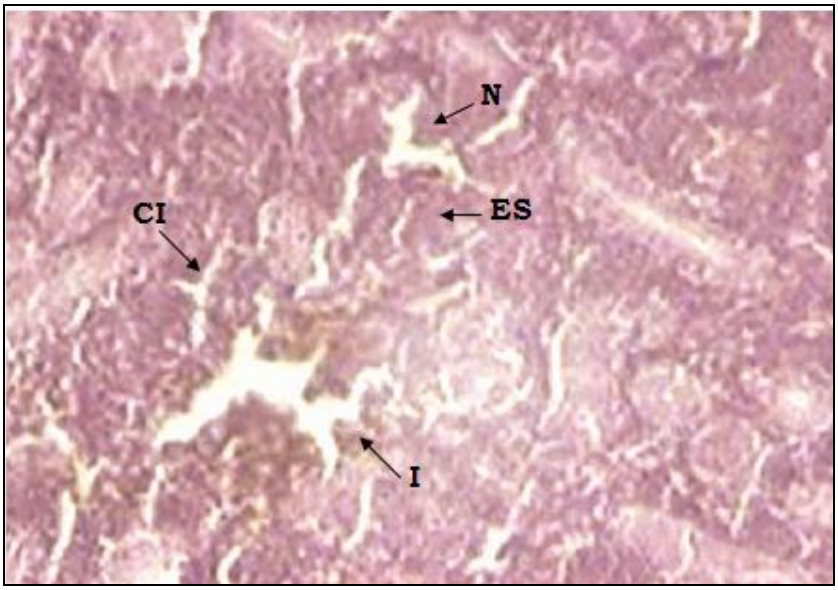

Figure 10: Kidney (28 days)