ISSN: 0976-3376

Available Online at http://www.journalajst.com

ASIAN JOURNAL OF SCIENCE AND TECHNOLOGY

Asian Journal of Science and Technology
Vol. 07, Issue, 05, pp.2971-2979, May, 2016

RESEARCH ARTICLE

HISTOPATHOLOGICAL EFFECTS ON THE FRESHWATER FISH, *LABEO ROHITA* WHEN EXPOSED TO THE PESTICIDE MONOCROTOPHOS

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ARTICLE INFO

Article History:
Received 17th February, 2016
Received in revised form
19th March, 2016
Accepted 22nd April, 2016
Published online 30th May, 2016

Key words:

Pesticide,
Histology, Toxicity,
Organs, *Labeo rohita.*

ABSTRACT

The assessment of the ecotoxicological risks caused by pesticides to ecosystems is based on data on the toxicity and effects of pesticide preparations to non-target organisms. Fish are among the group of non-target aquatic organisms. Responses of aquatic organisms are broad-ranged depending on the toxic compound, exposure time, water quality and the species. Monocrotophos is one of the organophosphorous pesticide used in this study. The median lethal concentration (LC 50) of MC to fish *L. rohita* for 96 h was found to be 45.1 ppm. In sublethal concentration(1/10th of LC 50 96h value, 4.51ppm) fishes were exposed for 24, 48, 72 and 96 hrs, 10 days, 20 days and 30 days. Histopathological lesions were noted in the gills, liver and kidney when compared to the control.

INTRODUCTION

The contamination of surface waters by pesticides used in agriculture is a problem of worldwide importance (Sibley and Kaushik, 1991). The impact of ecosystem by different chemical pollutants such as pesticides, the poisoning by pesticides from agricultural fields is a serious water pollution problem and its environmental .Long term and short term effect may result in the incidence of toxicity of fish and other aquatic life forms (Edwards, 1973).The environmental pollution due to extensive usage of the pesticides, herbicides and ammonical fertilizers without proper management has far reaching effects on the survival potential of aquatic animals, for some of these toxic chemicals may persist in the environment for longer periods and show damage at histological level (Roy *et al*., 2006). The assessment of the ecotoxicological risks caused by pesticides to ecosystems is based on data on the toxicity and effects of pesticide preparations to non-target organisms. Fish are among the group of non-target aquatic organisms (Velisek *et al*., 2009). Histopathological studies have been conducted to help establish causal relationship between contaminant exposure and various biological responses (Schwaiger *et al.*, 1996).

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Histopathological investigations have proved to be a sensitive tool to detect direct effects of chemical compounds within target organs of fish in laboratory experiments and useful to evaluate the pollution potential of pesticides since trace levels of pesticides, which do not cause animal mortality over a given period, are capable of producing considerable original damage.

MATERIALS AND METHODS

Collection and Maintanence of Fish

Fingerlings of the freshwater fish, *Labeo rohita* ranging in weight from 4g to 8g and measuring (4cm to 6cm in length) were procured from Aliyar Fish farm, Tamilnadu, India. The procured bulk samples of *Labeo rohita* 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°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. 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). 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 method of APHA, (2005); temperature 27.2 ± 0.9 (^{o}C), pH 7.1 ± 0.1, dissolved oxygen 5.4 ± 0.4 (mg /l), total hardness 180 ±1.9 (mg /l), salinity 0.3 ± 0.1 (ppt). Continuous artificial aeration was maintained throughout the acclimation and exposure periods.

Toxicant

Monocrotophos is one of the organophosphorus insecticides extensively used in agriculture and animal husbandry (Rao, 2004). Monocrotophos is a brownish yellow liquid with a sharp smell that irritates the eyes and skin. The IUPAC name is dimethyl (E)-1-methyl-2-(methyl-carbamoyl) vinylphosphate. Molecular formula is C7H14NO5P and molecular weight is 223.2.

Determination of 96 h LC_{50} value of MC

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 chemical (Jack de Bruijin *et al*., 1991).Batches of 10 healthy fishes were exposed to different concentrations of pesticide, Monocrotophos 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, P^{H}, 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 24hrs upto 96hrs.It was found as for 48hrs, using probit analysis method (Finney, 1971).From the stock solution various sublethal concentrations were prepared for bioassay studies.

Sublethal toxicity

Seven groups of fishes were exposed to ($1/10^{th}$ of the pesticide 'Monocrotophos' for 24, 48, 72 and 96 hrs, 10 days, 20 days and 30 days. 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 for further analysis. 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 Monocrotophos pesticide. At the end of exposure period, fish were randomly selected for histopathological examination. Tissues of gills, liver, kidney and muscle 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 6u 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 Qx3, Intel Corporation, Made in China).

RESULTS

The exposure of the fresh water fish, *Labeo rohita* to 4.51 per cent of Monocrotophos pesticide. Monocrotophos pesticide 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

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 in 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. The secondary lamellae on both sides were highly vascularised and covered by a layer of epithelial cells with uniform interlamellar spaces ().

Short term exposure

When the fish was exposed for 24 hours to the short term exposure of Monocrotophos pesticide, there was degeneration of epithelial lining ().After 48 hours of exposure, degeneration changes in the secondary lamellar of gill()was noted. After 72 hours of exposure, there was fusion of secondary lamellae with irregular lamellar spaces ().After 96 hours of exposure, structural alterations such as epithelial proliferation, lamellar fusion and necrosis were observed ().

Long term exposure

When the fish was exposed for 10 days to long term exposure of Monocrotophos pesticide considerable degenerative changes were observed. The secondary lamellae showed necrosis at the basal region. It showed congestion with infiltration by the chronic inflammatory cellular exudates ().After 20 days, papillary formation with dilated the vasculature. The infiltration was found with chronic inflammatory cells (). After 30 days, the damage became more noticeable leading to collapsed secondary lamellae ().

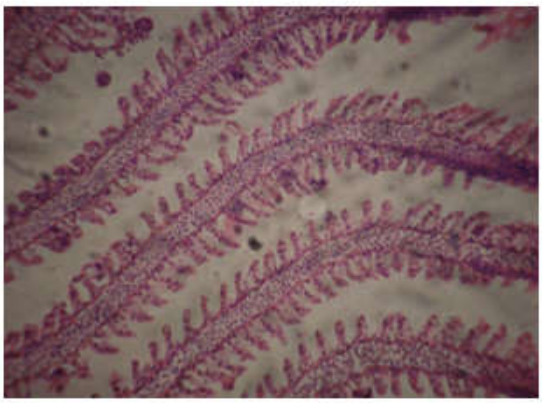
Liver Histology

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. Bile canaliculus was centrally located in each cord. Fairly large quantities of lipids and glycogen were observed in the hepatocyte cytoplasm ()

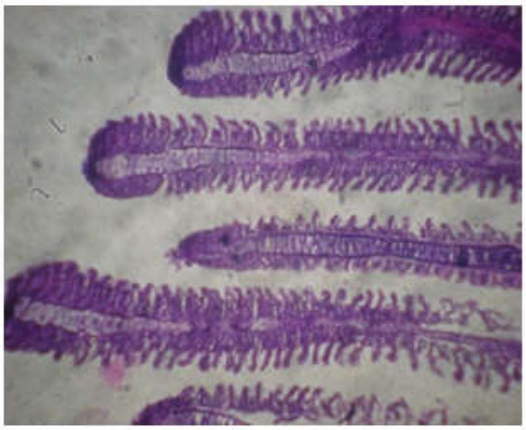
Short term exposure

When the fish was exposed for 24 hour to the short term exposure of Monocrotophos pesticide, the liver showed symptoms of general necrosis and degeneration of hepatocytes (). After 48 hours of exposure, clumping of nucleus, fatty degeneration () was noted. After 72 hours of exposure, hepatic cords showed evidence of cloudy swelling.

PL - Primary lamellae
SL - Secondary lamellae
LS - Lamellar space
ILS - Inter lamellar space
SA - Supporting axis

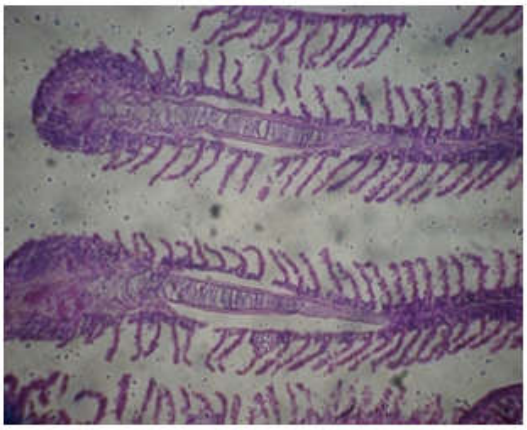


Control Gill section of *Labeo rohita*



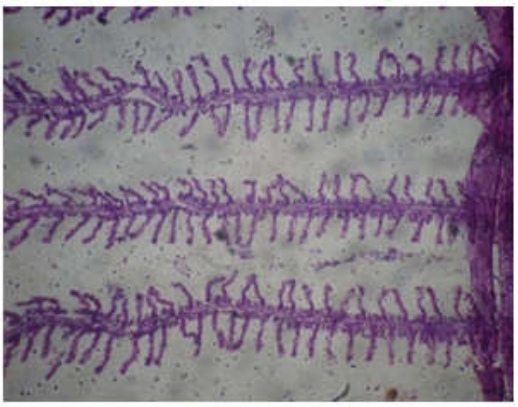
24 hours

SL - Secondary lamellae
ILS - Inter lamellar space
DEL - Degeneration of Epithelial lining



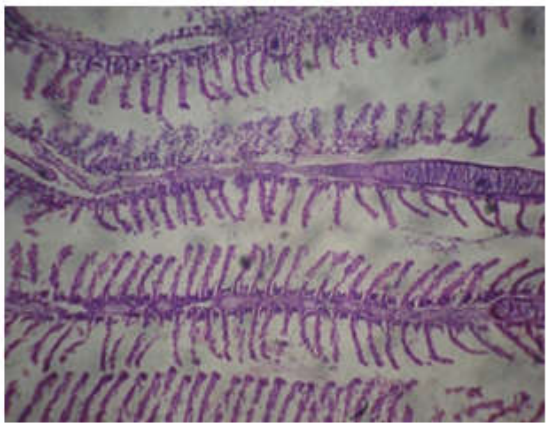
48 hours

PL - Primary lamellae
SL - Secondary lamellae
LS - Lamellar space
ILS - Inter lamellar space
DEL - Degeneration of Epithelial lining



72 hours

LS - Lamellar space
DEL - Degeneration of Epithelial lining
DS - Degenerated secondary lamellae

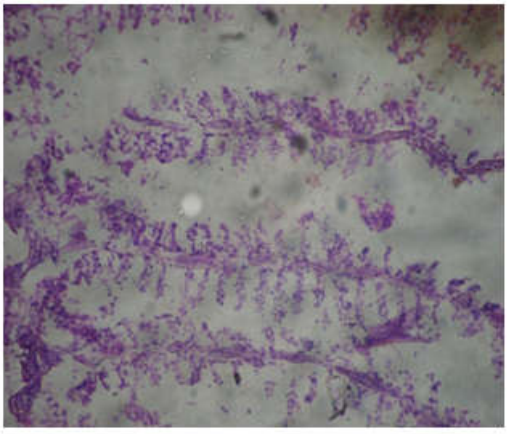


96 hours

LS - Lamellar space
DEL - Degeneration of Epithelial lining

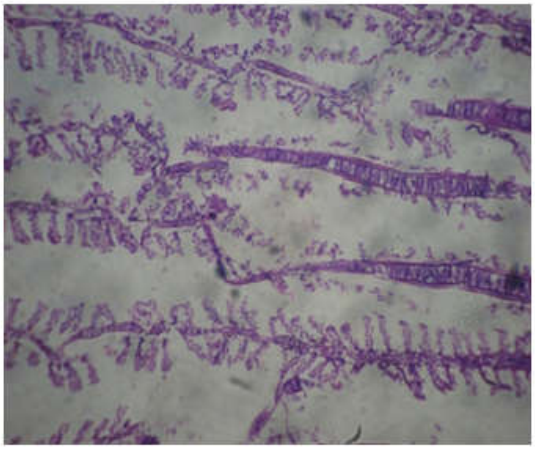
10 days

LS - Lamellar space
DEL - Degeneration of Epithelial lining
SL - Secondary lamellae
NS - Necrosis
EL - Epithelial lining



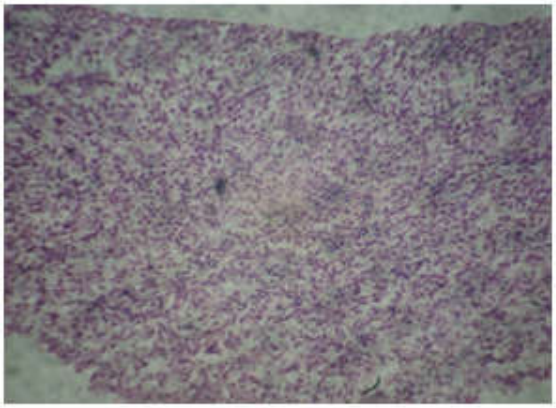
20 days

LS - Lamellar space
DEL - Degeneration of Epithelial lining
SL - Secondary lamellae
NS - Necrosis
ILS - Inter lamellar space



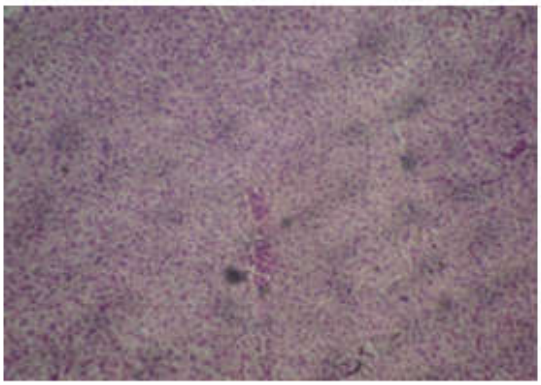
30 days

LS - Lamellar space
DEL - Degeneration of Epithelial lining
CSL - Collapsed Secondary Lamellae



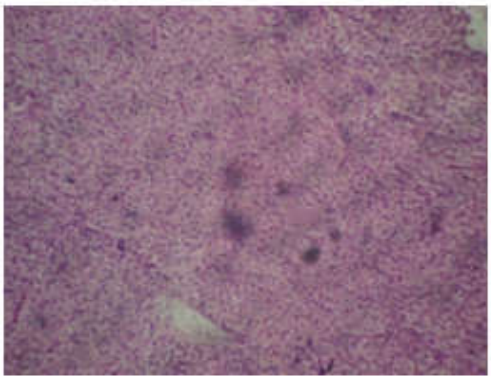
Control Liver section of *Labeo rohita*

HC - Hepatocyte cells
HCH - Hepatic cords
CEV - Central Efferent vein



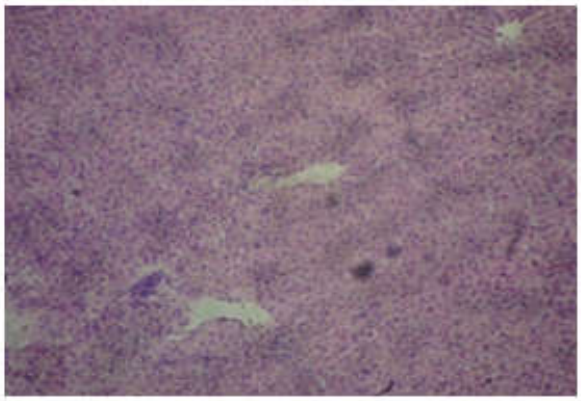
24 hours

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



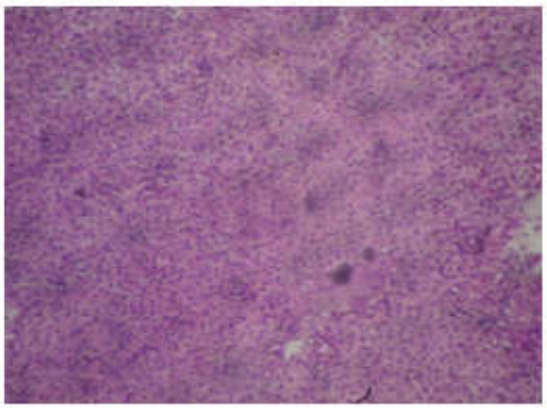
48 hours

DHC - Degenerated Hepatocyte Cells
HC - Hepatocyte cells
GC - Gilssen's capsule
CN - Clumping of Nucleus
FD - Fatty Degeneration



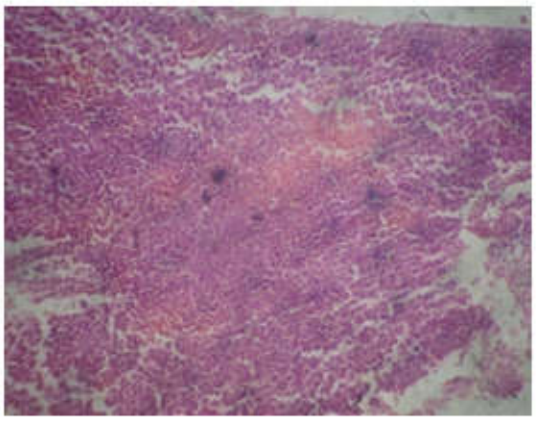
72 hours

HC - Hepatocyte cells
HCH - Hepatic cords
CEV - Central Efferent vein



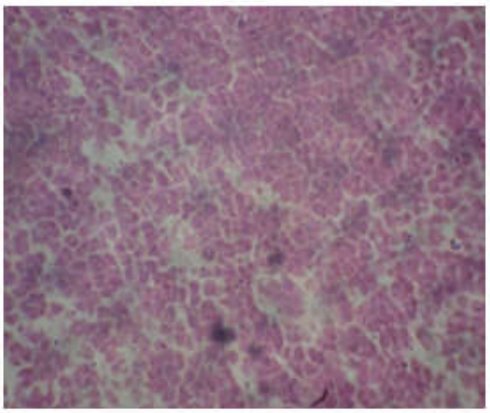
96 hours

HC - Hepatocyte cells
CEV - Central Efferent vein
GC - Gilssen's capsule



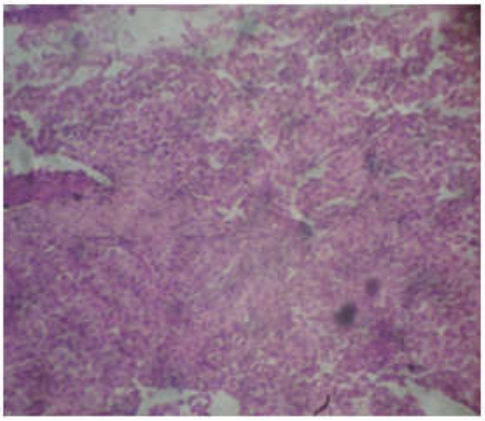
10 days

HC - Hepatocyte cells
HCH - Hepatic cords
GC - Gilssen's capsule



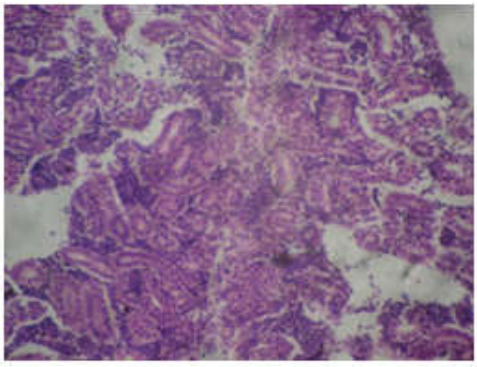
20 days

HCH - Hepatic cords
GC - Gilssen's capsule
VD - Vacuolar Degeneration
K - Karyolysis



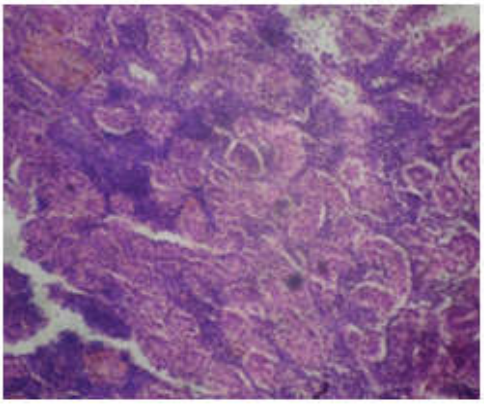
30 days

HC - Hepatocyte cells
HCH - Hepatic cords
GC - Gilssen's capsule
CSH - Cloudy Swelling of Hepatocyte



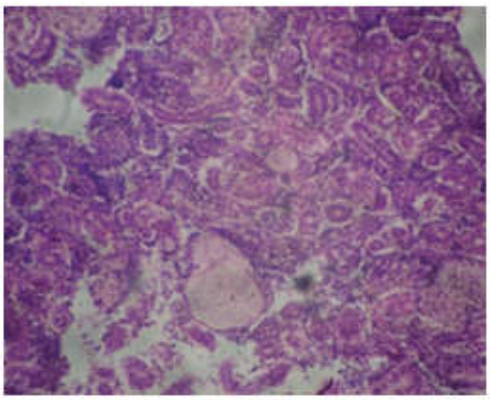
Control Kidney section of *Labeo rohita*

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



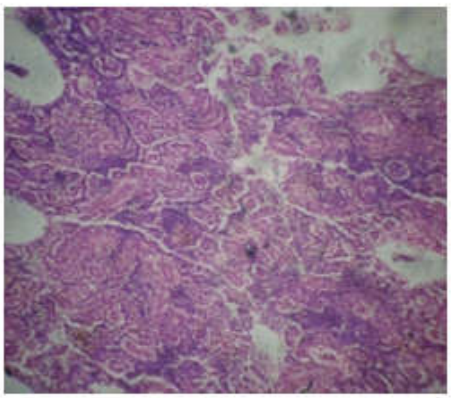
24 hours

GL – Glomeruli
LC - Lymphoid cells
PC - Parenchyma cells
BC - Bowman's capsule



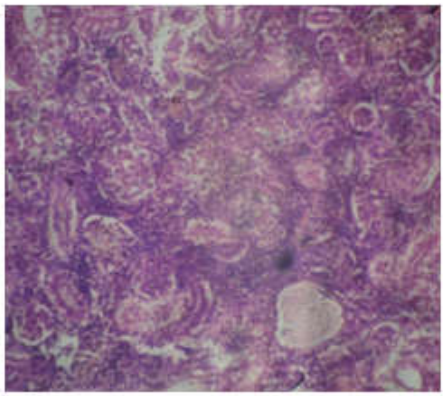
48 hours

PC - Parenchyma cells
BC - Bowman's capsule
T - Tubules
GL – Glomeruli



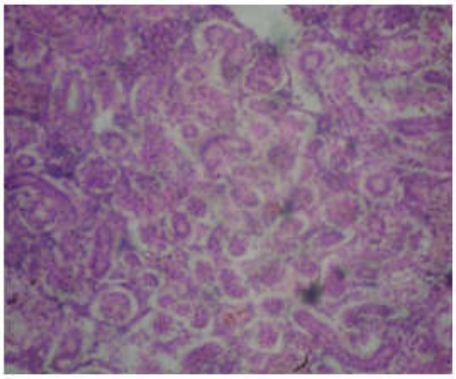
72 hours

LC - Lymphoid cells
PC - Parenchyma cells
BC - Bowman's capsule
SC - Shrunkened cells



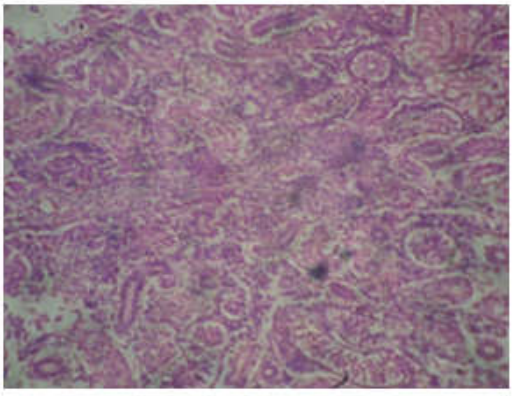
96 hours

BC - Bowman's capsule
SC - Shrunkened cells
SG - Shrunkened Glomerulus
TN - Tubules Nucleus



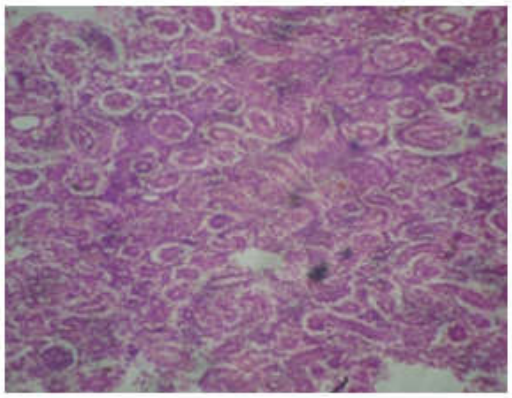
10 days

BC - Bowman's capsule
SC - Shrunkened cells
SG - Shrunkened Glomerulus
TN - Tubules Nucleus



20 days

BC - Bowman's capsule
SC - Shrunkened cells
SG - Shrunkened Glomerulus
TN - Tubules Nucleus



30 days

SC - Shrunkened cells
SG - Shrunkened Glomerulus

The central efferent vein and sinusoids were dilated (). After 96 hours of exposure, the parenchyma cells showed cloudy swelling. The Glissen's capsules were not thickened ().

Long term exposure

In 10 days exposure the pathological changes observed in the liver included degeneration of cytoplasm of hepatocytes, pycnosis of nucleus formation of vacuoles ().After 20 days of exposure, degeneration of hepatocytes and karyolysis () were noted. After 30 days of exposure, rupture in blood vessels and disposition of hepatic cords, cloudy swelling of hepatocytes, necrosis,portal triads were infiltrated with chronic inflammatory cells().

Kidney Histology

The kidney consisted of head and body kidneys. Head kidney, the anterior portion consisted of lymphoid tissues. 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. Mesangium filled the space between the loops of glomerular capillaries ().

Short term exposure

When the fish was exposed for 24 hours to the short term exposure of Monocrotophos pesticide, the kidney showed degenerative changes with dilated glomeruli and Bowman's capsules (). After 48 hours of exposure highly degenerative changes were found in haemopoietic tissues (). After 72 hours of exposure severe necrosis and moderately dilated renal tubules with infiltration of parenchyma by inflammatory cells (). After 96 hours of exposure, shrunkened glomerulus and nephritic changes were seen, Bowman's capsules were dilated ().

Long term exposure

When the fish was exposed for 10 days to long term exposure of Monocrotophos pesticide, the kidney showed intercellular space and shrunkened cells were noted (). After 20 days of exposure, tubular necrosis, shrunkened glomerulus were observed ().After 30 days of exposure, the glomeruli and Bowman's capsules were dilated. Severe pathological changes included necrosis, cloudy swelling of renal tubules, disintegration of interstitial tissues and pycnotic nuclei ().

DISCUSSION

Gill Histology

Balasubramanian *et al*. (1999) have reported degeneration of mucus cells of the gill epithelium, hypertrophy, necrosis and separation of epithelium due to edema in *Oreochromic mossambicus* under ambient urea stress. Piyanut peebua *et al*. (2008) reported clubbing at the tip of the secondary lamellae, hypertrophy and hyperplasia in gills of *Oreochromis niloticus* exposed with Alachlor. Erkmen *et al*. (2000) studied the histopathological effects of Cyphenothrin on the gills of *Lebistes reticulates*. The results showed Cyphenothrin lifts the epithelial layer from gill lamellae and form necrosis, degeneration of secondary lamellae due to odema, shortening of secondary lamellae.

Liver Histology

Tilak *et al*. (2001) reported on the effect of pesticide on the *Ctenopharyngodon idellus* liver and found degenerative of hepatocytes formation of vacuoles, rupture in blood vessels, necrosis and disappearance of the hepatocyte wall and disposition of the hepatic cords. Hyperplasia, vacuolation, disintegrated blood vessels, disrupted hepatocytes, focal coagulative necrosis disorganized hepatic canaliculi in *Labeo rohita* exposed to Cypermethrin (Sarkar *et al.*, 2005). Atif *et al*. (2009) have found rapid and continued destruction of erythrocytes with increased haemolysis and damage of the iron metabolism.

Kidney Histology

Das *et al.* (2000) have observed capillary tuft of the glomeruli shrunken and Bowman's capsule thickened due to the effect of chemical pollutants.Weber *et al*. (2003) have found the occurrence of partly occluded renal tubule and fatty degeneration in most of the specimens. These pathological conditions appear to be consequence of dead and dying epithelial cells. Velmurugan *et al*. (2007) have reported that the necrosis of the renal tubules affects the metabolic activities and promotes metabolic abnormalities in fish, *Cirrhinus mrigala* exposed to Fenvalerate.

Conflict of Interest

None declared.

Acknowledgement

Authors are gratefully thankful to staff members for their valuable suggestions and Head of the Department of Zoology for providing facilities to carryout this research work successfully.

REFERENCES

Anon, 1975. Recommendations of the committee on methods for toxicity tests with fish, macro-invertebrates and amphibians. EPA, *Oregon*.61.

Atif, M., Naggar. EL., Soaad, A., Mahmoud and Safaa, I. 2009. Bioaccumulation of some heavy metals and histopathological alterations in liver of *Oreochromis niloticus* in relation to water quality at different localities along the river Nile, Egypt. *World Journal of Fish and Marine Sciences.* 1(2):105-114.

Balasubramanian, P., Saravanan,I.S. and Palaniappan, M. K. 1999. Biochemical and histopathological changes in certain tissues of O*reochromis mossambicus* (Trewaves) under ambient urea stress.*Bull.Environ.Contam.Toxicol*., 63(1):117-124.

Das, B. K., Mukherjee, S. C. and Murjani, G. 2000. Histopathological studies on the myxoboliasis of *Cirrhinus mrigala*. *Indian J.fish*.47(1):61-64.

Edwards, C. A. 1973. In Environmental pollution by Pesticides. *Plenum press*.542.

Erkmen, B., Caliskan, M. and Yerli, S. V. 2000. Histopathological effects of Cyphenothrin on the gills of *Lebistes reticulates*. *Veet.Hum.Toxicol*., 42(1):5-7.

Finney, D. J. 1971. Probit analysis, 3rd edition, *(London:Cambridge University Press)*. 20.

Humason, G. L. 1962. Animal tissue technique III (ED) W. H.Freeman and Co., San Fransisco. *International Journal of Pharmaceutical and Biological Archives*. 2 (4):1215-1217.

Jack de Bruijin, Eddy yedema, Willen senior and Joop Heimeus. 1991. Lethal body burdens of four Organophosphorus pesticides in the guppy (Poecilia reticulata). *Aquatic Toxicology*, 20:111-122.

Piyanut peebua, Maleeya Kruatachue prayad, Pokethitiyood. and Sombal Singhakaew. 2008. Histological alterations of Nile tilapia, *Oreochromis niloeicus* in acute the subchronic Alachlor exposure.*J.Environ.Biol*., 29 (3):325-331.

Rao, J V. 2004. Effects of monocrotophos and its analogs in acetylcholinesterase activity's inhibition and its pattern of recovery on euryhaline fish, *Oreochromis mossambicus*. *Ecotox. Environ Safe.* 59:217–222.

Roy, P. K. and Datta Munshi, J. S. 2006: Malathion induced structural and morphometric changes of gills of a fresh water major carp, *Cirrhinus mrigala* (Ham). *J. Environ. Biol*., 12:79-87.

Sarkar, B., Chatterjee, A., Adhikari, S. and Ayyappan, S. 2005. Carbofuran and cypermethrin induced histopathological allterations in the liver of *Labeo rohita* (Hamilton) and its recovery.J.*Appl.Ichthyol*., 21: 131-135.

Schwaiger, J., Fent, K., Stecher, H., Ferling, H. and Negele, R. D. 1996. Effects of sublethal concentrations of Triphenyltinacetate on rainbow trout *(Oncorhynchus mykiss*). *Arch Environ Contam Toxicol.,* 30: 327-34.

Sibley, P. K. and Kaushik, N. K. 1991. Toxicity of microencapsulated Permethrin to selected nontarget aquatic invertebrates. *Archives of Environ. Contamination and Toxicology*. 20:168–176.

Tilak, K. S., Veeraiah, K. and Yacobu, K. 2001. Studies on Histopathological changes in the gill, liver and kidney of *Ctenopharyngodon idellus* (Valenciennus) exposed to technical Fenvalerate and EC 20% *Poll.Res*., 20(3): 387-393.

Velisek, J., Stastna, K., Sudova, E., Turek, J. and Svobodova, Z. 2009. Effects of subchronic Simazine exposure on some biometric, biochemical, hematological and histopatological parameters of the common carp (*Cyprinus carpio* L.) *Neuroendocrinology Letters*, 30 (1): 236-241.

Velmurugan, B., Selvanayagam, M., Cengiz, E. I. and Unlu, E. 2007. The effects of fenvalerate on different tissues of freshwater fish, *Cirrhinus mrigala*. *J. Environ .Sci*., 42:157-163.

Weber, L. P., Higgins, P. S., Carlson, R. I. and Janz, D. M. 2003. Development and validation of methods for measuring multiple biochemical indices of condition in juvenile fishes. *Journal of fish Biology*. 63:637-658.

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