ISSN: 2319-6505

Available Online at http://journalijcar.org

International Journal of Current Advanced Research
Vol 5, Issue 4, pp 730-733, April 2016

International Journal of Current Advanced Research

ISSN: 2319 - 6475

RESEARCH ARTICLE

TOXICITY EFFECT OF THE DETERGENT TIDE ON THE HISTOPATHOLOGY OF THE FRESHWATER FISH *CIRRHINUS MRIGALA*

Vasanthi J.,[1] and Binukumari S.,[2]

[1]Department of Zoology, Nirmala College for women, Coimbatore-18
[2]Department of Zoology, Kongunadu Arts and Science College, Coimbatore-29

A R T I C L E I N F O

Article History:

Received 06th January, 2015
Received in revised form 14th February, 2016
Accepted 23rd March, 2016
Published online 28th April, 2016

A B S T R A C T

Histopathological alterations can be used as indicators for the effects of various anthropogenic pollutants on organisms and are reflect of overall health of the entire population in the ecosystem. The fingerlings of *Cirrhinus mrigala* were procured from fish farm and acclimatized in the laboratory for one week. The LC_{50} value was determined and the experimental fishes was exposed to sublethal concentration. After the exposure periods for 10, 20 and 30 days the fishes were sacrificed and the organs like gill, liver and kidney were examined for histopathological alterations. Degeneration of primary and secondary lamellae in gills, karyolysis, degeneration of hepatocytes, clumping of nucleus in liver and tubular necrosis, shrunken glomerulus in the kidney was observed. From the present study the changes observed in tissues of different organs of fishes were evident to show the effects of aquatic pollution.

INTRODUCTION

The toxicity of any pollutant is either acute or chronic. The chronic studies include both histochemistry and pathology. Although toxicant impairs the metabolic and physiological activities of the organisms, physiological studies alone do not satisfy the complete understanding of pathological conditions of tissue under toxic stress. Hence it is useful to have an insight into the histological analysis.

The extent of the severity of tissue damage is a consequence of the concentration of the toxicant and is time dependent. Also the severity of damage depends on the toxic potentiality of a particular compound or pesticide accumulated in the tissue. (Jayantha Rao 1984).

In fish, gill is the first organ to which the pollutant comes into contact. Hence, it is more vulnerable to damage than any other tissue. Previous histopathological studies in fish exposed to pollutants have shown that fish gills are primary markers for aquatic pollution (Bernet 1999).

Histopathological effects of sublethal concentrations of dichlorvos on the gill and liver of *Cirrhinus mrigala* was reported by Babu Velmuguran *et al* (2009). Swarna kumari and Tilak (2010) reported vestiges of hydropsy, vascular degeneration, bulging and severe necrotic changes in gill of the fish *Ctenopharyngodon idella* exposed to an organo phosphate, Nuvan, 76% EC.

MATERIALS AND METHOD

ANALYTICAL TEST FOR WATER CHEMISTRY

The tap water free from contaminants was used as dilution water for the present study. The physico-chemical analyses of water used in the experiments were carried out using the method of APHA (1998).

Physico-chemical parameters of the tap water used for the present study are as follows; Temperature 27.2 ± 0.9 (^{o}C), pH 7.1 ± 0.1, Dissolved O_2 5.9 ± 0.4 (mg/I), Alkalinity 148 ± 0.7 (mg/I), Salinity 0.4 ± 0.1 (ppt), Total Hardness 190 ± 1.9 (mg/I), Calcium 132 ± 1.1 (mg/I), Magnesium 65 ± 0.2 (mg/I).

PROCUREMENT AND MAINTENANCE

The fingerlings of the freshwater fish, *Cirrhinus mrigala* ranging in weight from 3g to 8g and measuring 4cm to 8cm in length) were procured from "Tamil Nadu Fisheries, Department corporation" Mettur, Salem District. 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 photoperiod for one week in large plastic containers at (26 ± 5 ^{o}C). The tank was previously washed with potassium permanganate to prevent any fungal infection. The fishes were maintained in dechlorinated tapwater of the quality used in the test and water was renewed everyday to provide freshwater, rich in oxygen.

Continuous artificial aeration was maintained throughout the acclimation and exposure periods. During this period the fish

were fed with mixture of oilcake and rice bran. 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).

Technical grades of Tide an detergent was used in this investigation. Detergent Tide contains Alkyl Sulfate, Borax, citric acid, Diethylenetriamine pentaacetate (Sodium salt), Ethanolamine, FD & C Blue 1,Glycerin, Hydrogen peroxide, Liquitint™ Blue, Mannanase, Nonanoyloxybenzenesulfonate, polyethylene oxide, Sodium polyacrylate and Trimethoxy Benzoic Acid.

EVALUATION OF MEDIAN LETHAL CONCENTRATION (Lc50)

The concentrations of the pollutant at which 50 percent of the test animals die during a specific test period or the concentration lethal to one half of the test population is referred to as median lethal concentration (LC_{50}) or median tolerance 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 detergent Tide 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 10-100 mg were chosen and the number of dead or affected fishes in each setup 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 10-50 mg 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 72 hrs. It was found as 36mg for 48 hrs, using probit analysis method (Finney, 1971). From the stock solution various sub-lethal concentrations were prepared for bioassay study.

Three groups of fishes were exposed to 3.6 mg ($1/10^{th}$ of 48 hrs LC_{50} value) concentration of the detergent 'Tide' for 24, 48 and 72 hrs 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 liver, gill, muscle, brain and kidney were dissected and removed. The tissues (10mg) were homogenized in 80% methanol, centrifuged at 3500 rpm for 15 minutes and the clear supernatant was used for the analysis of different parameters.

HISTOPATHOLOGY

Freshwater fish *Cirrhinus mrigala* were exposed for 10 days, 20 days and 30 days to a sublethal concentration (36 mg) of detergent Tide. At the end of exposure period, fish were randomly selected for histopathological examination.

Tissues 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 Qx3, Intel Corporation, Made in China).

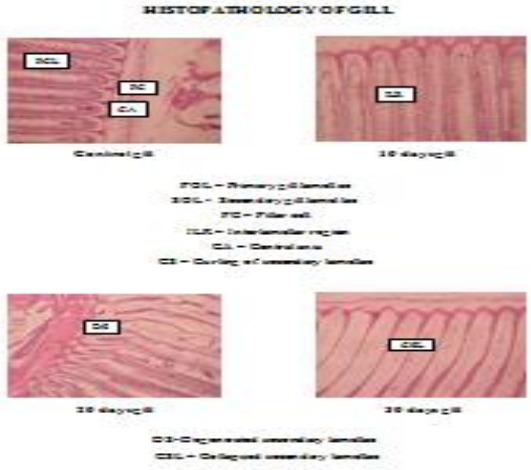

Figure 1

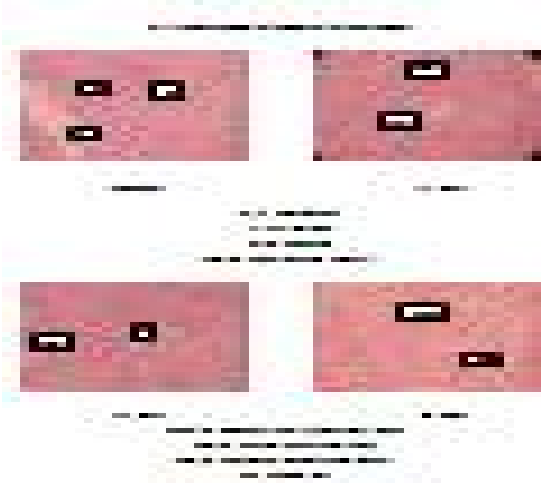

Figure 2

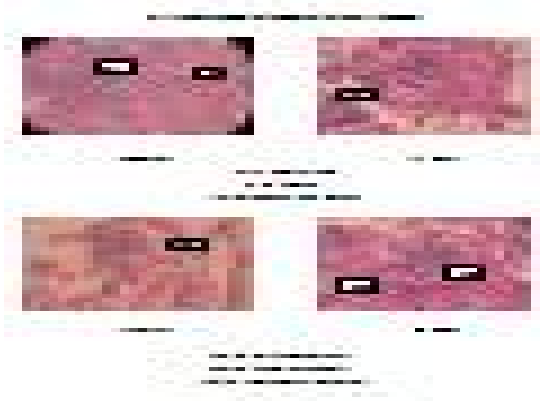

Figure 3

RESULTS AND DISCUSSION

The exposure of the freshwater fish *Cirrhinus mrigala* in the sublethal concentration of detergent Tide (36 mg) for 10, 20, 30 days led to the formation of histopathological lesions of

varying intensites on the gill tissues and internal organs like liver and kidney.

In present investigation the histopathological changes in gills of *Cirrhinus mrigala* in normal condition and exposed to sublethal concentrations of detergent at selected periods of exposure have been observed in present study.

Unexposed fish gill histology revealed the presence of primary and secondary gill lamellae, pillar cell, inter lamellar region and central axis (ph.m.1) The fish exposed to Tide for 10 days exposure period, exhibited marked histopathological changes, such as curling of secondary lamellae (ph.m.2.) After 20 days exposure, degenerated secondary lamellae were noted. (ph.m.3.) The severity of the damage become more noticeable after 30 days of exposure leading to collapsed secondary lamellae (ph.m.4).

The histopathological changes in the liver of freshwater fish *Cirrhinus mrigala* were occurred after long term exposure to detergent Tide. In control the liver tissues of *Cirrhinus mrigala* is composed with hepatocyte, Sinusoid and Nucleus (ph.m.5) Fish exposed to Tide showed clumping of nucleus, fatty degeneration during tenth day exposure (ph.m.6.) After 20 days exposure, cloudy swelling of hepatocyte, vacuolar degeneration were noted (ph.m.7). The pathological lesions were more drastic and widespread after 30 days of exposure, thereby degeneration of hepatocytes and karyolysis were observed (ph.m.8).

In the present study it has been observed that the kidney of *Cirrhinus mrigala* revealed certain histopathological changes on exposure to Tide for longer durations of 10, 20 and 30 days. The kidney tissue of control fish is comprised of glomerulus and tubule (ph.m.9). The fish exposed to Tide for 10 days exposure period, showed intercellular space (ph.m.10). After 20 days exposure, shrunken of cells were noted (ph.m.11). Tubular necrosis, shrunkened glomerulus were some of the degenerative changes noticed in fishes exposed to 36 mg Tide for 30 days (ph.m.12).

Histological changes provide a rapid method to detect effects of irritants, especially chronic ones, in various tissues and organs (Bernet *et al*., 1999).

Water pollution induces pathological changes in fish. As an indicator of exposure to contaminants, histology represents a useful tool to assess the degree of pollution. Tissue damages brought about by water-borne pollutants can be easily observed because the fish gills come into immediate contact with the environment.

In the present study a comparative histological observation of gill, liver and kidney of the control and treated fishes clearly showed that the detergent Tide has caused histopathological changes in these organs (Ph.m. 1-12).

Fish gills are vulnerable to pollutants in water because of their large surface area and external location. However the gills perform numerous functions which include respiration, excretion of nitrogenous waste products and acid base balance.

In the present work exposure to Tide at 36mg concentration level induced many pathological changes in the gills of *Cirrhinus mrigala* such as curling, degenerated and collapsed secondary lamellae (Ph.m.1-4).

Histopathological changes in the gills of fishes due to pesticides and other contaminants have been reported by several authors. Haemorrhage at primary lamellae, fusion of secondary lamellae, epithelial necrosis and hypertrophy of epithelial cells have been reported in fish gills exposed to various kind of pesticides (Vijayalakshmi and Tilak, 1996; Erkmen *et al*., 2000; Cengiz and Unlu, 2002; Rao *et al.,* 2006; Jayachandran and Pugazhendy, 2009).

According to Eller (1971a) the epithelial layer of secondary lamellae of gill of fish forms a barrier between the fish blood and surrounding water. Gaseous exchange needed to sustain life which takes place through this barrier and any thickening induced by physical, chemical or biological agents hinders the respiratory function of this organ.

Fish liver is regarded as a major site of storage, biotransformation and excretion of toxicant. Alterations in the liver may be useful as markers that indicate prior exposure to environmental stressors.

In the present study, liver tissue of fish *Cirrhinus mrigala* exposed to detergent Tide (36mg) for longer durations was found to show clumping of nucleus, fatty degeneration, vacuolar degeneration, karyolysis and degeneration of hapatocytes.(Ph.m.5-8).

Similar view has been advocated by many authors. Degeneration of cytoplasm with pyknosis of nuclei, cloudy swelling, atrophy, vacuolization have been reported by Narayan and singh, (1991); Cengiz *et al*., (2001); Fanta *et al*., (2003). Babu Velmurugan *et al*., (2009) have observed hepatic lesions in the liver tissues of fishes such as cloudy swelling of hepatocytes, congestion, vacuolar degeneration, karyolysis and nuclear hypertrophy when exposed to dichlorvos.

The severe histopathological lesions observed in liver tissue could be possibly due to the sudden withdrawal and utilization of stored glycogen from the liver cells to meet the energy demand under toxic conditions.

The posterior kidney of freshwater fishes is adaptated to produce diluted urine and it has little participation in ion or acid-base balance.

In the present study, renal tissue of the fish *Cirrhinus mrigala* under detergent Tide revealed certain pathological changes such as shrunken of glomerulus and tubular necrosis (Ph.m.9-12). Many reports on the pathological changes in the renal tubules of the fish exposed to various pesticides have been reported by (Saxena, 1993; Teh *et al*., 1997).

Kumar Ravinder (2000) have observed various changes in the nucleus of all tubular cells such as pyknotic nucleus and granular cytoplasm in the fish *Channa punctatus* on exposure to ammonia. Histological alterations such as tubular epithelium, necrotic renal tubules and glomerulus were analysed by Ortiz *et al*., (2003) and Staicu Andrea cristina *et al*., (2008).

Fishes receive the vast majority of postbranchial blood and because of that we expect renal lesions when the fish are exposed to pollutants. The changes in the size of cells and the narrow lumen could be the consequence of changes in kidney function. The fact that the physiology of the tubule cells is affected may be noticed from the nuclear changes too.

CONCLUSION

Histological studies were carried out in the tissues gills, liver and Kidney of the *Cirrhinus mrigala* exposed to 36mg of detergent Tide. Degenerative changes were noticed only after long term exposure. The result of the present study indicates that the alterations of pathological changes in different tissues may lead to fish morbidity and mortality.

References

1. Jayantha Rao K. (1984) Histopathology as a diagnostic tool in evaluation of toxicity, *environ. Toxicol*. Selected lectures and methods, S.V. University, Tirupathi, India.
2. Bernet D. Scmidt H.Meier W.Burkhardt-Holm P, Wahli T (1999). Histopathology in fish: proposal for a protocol to assess aquatic pollution. *J.Fish Dis*.22 (1):25-34.
3. Babu Velmurugan, Mariadoss selvanayagam, Elif Ipek Cengiz and Erhan Unlu (2009).Histopathological changes in the Gill and Liver Tissues of Freshwater fish, *Cirrhinus mrigala* exposed to Dichlorvos. Braz. Arch. Biol. Technol, 52 (5) :1291-1296.
4. Swarnakumari. R and Tilak, K.S. (2010), Histopathological changes observed in the gill tissue of the fish *Ctenopharyngodon idella* exposed to an organo phoosphate, Nuvan 76% Ec. *J. Ecotoxicol. Environ. Monit*. 20(3): 217-223.
5. APHA, (1998). In: Standard methods for the examination of water and waste water. 20th edn., *Am.Pub.HHh.Assoc.Washington*. DC, p.566.
6. Anon (1975) Committee on methods of toxicity tests with fish macro invertebrates and amphibians. EPA. Oregon, p.61.
7. Jack de Bruijis, 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.
8. Finney, D.J. (1971). Probit analysis, 3rd edition, (London: Cambridge University press). Pg.20.
9. Humason, G.L. (1962). Animal tissue technique III (ED) W.H. Freeman and Co., San Fransisco.
10. Vijayalakshmi, S. and Tilak, K.S. 1996. Effect of pesticides on the gill morphology of *Labeo rohita. J. Ecotoxicol. Environ. Monit*. 6(1): 59-64.
11. 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.
12. Cengiz, E.I & Unlu.E, (2002), Histopathological changes in the gills of mosquito fish, *Gambusia affinis* exposed to Endosulfan. *Bull. Environ. Contam. Toxicol*, 68, 290-296.
13. Rao, J.V. Begum,G., Jakka, N.M, Srikanth, K, Rao, R.N. (2006), Sublethal effects of profenofos on locomotor behavior and gill architecture of the mosquito fish, *Gambusia affinis. Drug. Chem. Toxicol*., 29, 255-267.
14. Jayachandran. K and Pugazhendy.K (2009). Histopoathological changes in the Gill of *Labeo rohita* (Hamilton) Fingerlings exposed to Atrazine. *American-Eurasian Journal of Scientific Research.* 4(3): 219-221.
15. Eller L.L (1971a) Gill lesions in freshwater teleosts.In: The Pathology of fishes.Eds: Riubelin W E, Migaki G Univer.Wis.Press.p.305-330.
16. Narayan, A. S., Singh, B.B (1991): Histopathological lesions in *Heteropneustes fossilis* subject to acute thiodan toxicity. Acta Hydrochem.Hydrobiol. 19, 235-243.
17. Cengiz, E.I, Unlu, E and Balci K, (2001). The histopathological effects of thiodan^{R} on the liver and Gut of mosquito fish, *Gambusia affinis. Journal Environmental Science Health* 36 (1), 75-85.
18. Fanta, E.; Rios, F.S.; Romao, S.; Vianna, A. C. C.; Freiberger, s. (2003). Histopathology of the fish *Corydoras paleatus* contaminated with sublathal levels of organophosphorus in water and food.*Ecotox.Environ. Safe.* 54,119-130.
19. Saxena and Meena (1993).Rogor toxicity in the intestine and Kidney of a freshwater teleost *Channa striatus* (Bloch).*Proc .Acad. Environ. Biol.*2 (2):225-228.
20. Teh, S.J., S.M.Adams and D.E.Hinton (1997). Histopathological biomarker in feral freshwater fish populations exposed to different types of contaminant stress.*Aquat.Toxicol.*37:51-70.
21. Kumar Ravindar (2000).Chronic ammonia induced histopathological changes in Indian subtropical fresh water murrel *Channa punctatus* (Bloch). *Polln.Res*. 19(4):611-613.
22. Ortiz, J.B., M. Luisa Gonzalez De Canales and Carmen Sarasquete (2003). Histopathological changes induced by lindane (HCH) in various organs of fishes. *Sci. Mar*. 67(1) : 53-61.
23. Staicu Andrea Cristina, Munteanu Maria Cristina, Dinischiotu Anca (2008). Malathion induced Histological modifications in gills and Kidney of *Carassius Auratus gibelic. Zootehnic si Biotehnologii*, 41(1).

****