

Histological Changes in Stomach of Fresh Water Fish *Danio aequipinnatus* After the Exposure of Newtech (Biopesticide).

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Abstract

In present investigation pathological effects produced by chronic exposure of biopesticide Newtech on stomach of fresh water cyprinid *Danio aequipinnatus* was studied. The chronic exposure of stomach to the pesticide newtech at sub-lethal concentration ($LC_{50}/10$) 0.1554 ppm for 7, 14, and 21 days respectively. Fishes showed sever histological changes in stomach. The degenerative changes induced fused microvilli, haemorrhage in the submucosa region, the outer membrane of microvilli are broken, vacuolisation, cell swelling are recorded. Such type of work can be helpful in understanding hazards of pesticides and pollution of pesticide by anthropogenic activity.

Index Terms - Histology, Stomach, *Danio aequipinnatus*, Pesticide, Newtech.

Introduction

Pesticides are applied on large scale to improve the yield production of agricultural crop. These pesticides are used to control agricultural pest. But, at the same time these pesticides also enter in lake, pond and river through rain water.it affects the life of living things of water chiefly to fish. Fishes are vulnerable to the toxic substances and bioaccumulation cause severe risk to the life. Pesticides after entering in the body of fish bring about histopathological and biochemical changes in different target and non-target organs³.The previous histo-pathological investigation of fish exposed to contaminants revealed that fish organs are efficient indicators of water quality². Chronic exposure of sub- lethal concentration of pesticide has been found to cause moderate to severe histopathological changes in the organs and tissues of fish⁷. The stomach is one of the primary organs of alimentary canal and plays an important role in the digestion of ingested food material for the growth and development of fish. Histologically the wall of the stomach consists of four layers: mucosa, submucosa, muscularis and serosa. The mucosal layer of stomach is generally simpler than that of higher vertebrate. However, the gastric glands of fish contain only one cell types that secrete both pepsinogen and hydrochloric acid⁶.The literature contains few studies of the histochemistry of mucosubstances in the fish stomach⁴. Most of the toxic agents like pesticides enter in to the digestive system through food material or water and affect the alimentary canal and digestive glands. It has direct contact

with the pollutants dissolved in the water but due to its contact with blood, it is indirectly affected¹². Biopesticides are usually considered as less toxic to non-target species and so little attention has been paid to assess the toxicity of them against non-target animals. Since the fishes are the top consumers of aquatic ecosystem and thus chance of pesticides accumulation are more in them. However, there has been little information on the impact of newtech on stomach of *Danio aequipinnatus*. In the present study *Danio aequipinnatus* has been selected as a test model animal. Since, the stomach is the first organ to come in to contact with contaminants, ultra structural changes of these organs were chosen as criteria for the sub-lethal action of Newtech was selected for the study. Thus, the objective of this study was to investigate the sub-lethal effect of Newtech to *Danio aequipinnatus*.

Materials and Methods

Similar sized, live and healthy adult fishes were bought by local fishermen from Rangavali River of Navapur Tahsil Dist. Nandurbar. Collected fishes were brought in to the laboratory in plastic container filled with reservoir water to avoid mortality. Fishes acclimatized for a time of 8-10 days in laboratory conditions in glass aquaria containing dechlorinated water. The aquaria water was aerated continuously through stone diffusers connected to a mechanical air compressor. Fishes were fed twice daily with fish food purchased from market. For the present study, matured adult fishes were exposed to Newtech at sub-lethal concentration ($LC_{50}/10$) 0.1554 ppm for 7, 14, and 21 days showed respectively. Three replicates of ten fishes for each concentration of the pesticides were used. In these aquaria water was replaced daily with fresh treatment of newtech pesticides. Each experiment was accompanied by its respective control.

At the end of exposure period, fish were randomly selected for histopathological examination. Tissues of stomach were isolated for 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. Stomach alone was processed by double embedding technique. Sections were cut at 6 μ thickness stained with Haematoxyline and Eosin, dissolved in 70% alcohol and were mounted DPX. The slides were observed under light microscope at 40X magnification and were then Photo-monographs were taken with the help of Lobamed binocular microscope attached to computer and analysed.

Results

Healthy fishes were kept for acclimatization exhibit a drastic behavioural change after exposure to Newtech. Histopathological changes observed in the stomach tissues of *Danio aequipinnatus*. Subjected to sub-lethal concentrations $1/10^{\text{th}}$ of the LC_{50} value i.e. 0.1554 ppm of Newtech for 7, 14 and 21 days.

A. Control stomach

The stomach wall was consists of mucosa, sub-mucosa, muscularis and serosa. The mucosal folds are composed of connective tissue cores covered by intestinal epithelium. Numbers of columnar epithelial cells

were observed in epithelial lining and closely resembled those of highly vertebrates. These cylindrical and tall cells had striated, microvilli (free brush border) and contain oval shaped nuclei in the central position of cells. Stomach goblet cells (mucous secreting cells) were interpreted among the columnar cells being more numerous along the slides rather than at the base of mucosal folds.

B) 0.1554 ppm 7 days exposure with Newtech :

When the slides of 0.1554 ppm of 7 days exposure ruptured epithelium in large amount were observed. Haemorrhage occurs in the microvilli region. Vacuolation in sub mucosal region. Outer membranes of microvilli were broken. Necrosis and vacuolisations were also observed in the region of lamina propria. Serosa was degenerated in some points. (Fig- B).

C) 0.1554 ppm 14 days exposure with Newtech:

When the slides of 0.1554 ppm of 14 days exposure shows more lesions than that of 7 days exposure. Epithelium was completely ruptured. Columnar cells ARE degenerated. Vacuolation also in sub mucosal region. Goblet cells completed degenerated (Fig- C).

D) 0.1554 ppm 21 days exposure with Newtech:

When the slides of 0.1554 ppm of 21 days exposure shows pyknosis nucleus in every cell of stomach region of *D.aequipinnatus*. Necrosis is more seen in this slide. Columnar cells are totally damaged. Columnar cells are degenerated. Lamina propria are enlarged and swelling up.

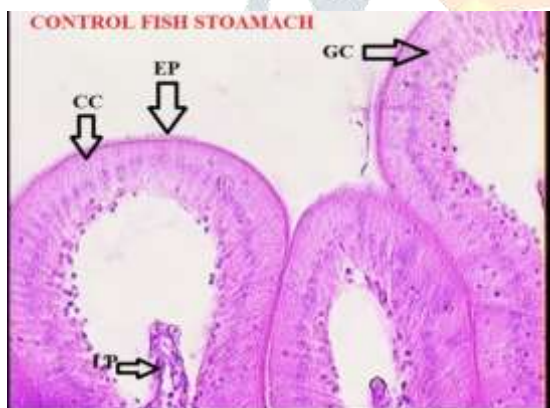


FIG - (A)

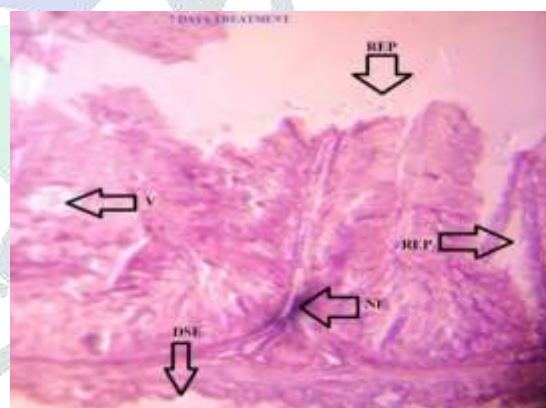


FIG - (B)

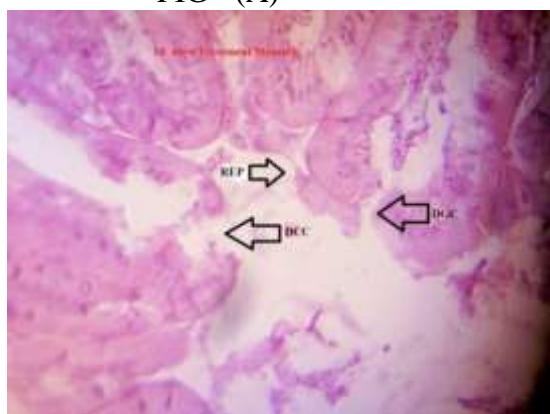


FIG - (C).

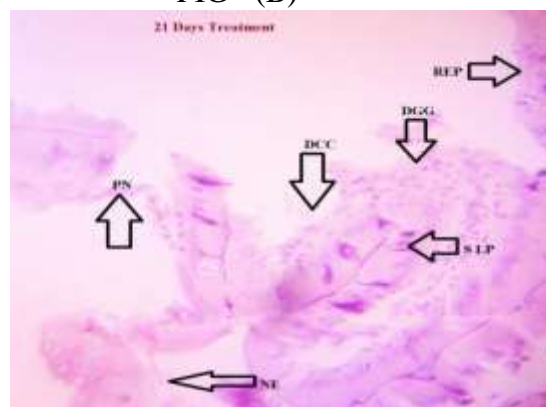


FIG - (D).

FIG- A. Control, Fig-B.7 Days Treat, Fig-C-14days, Treat. Fig-D-21 Days Treat.

GC-Goblet Cell. EP- Epithelium. LP-Lamina Propria. CC-Columnar Cells.

REP-Raptured Epithelium. DLP- Degenerated Lamina propria. V- Vacuolation. DGC-Damaged goblet cell. DCC- Damaged columnar cell. DSE- Damaged Serosa. PN-Pyknosis nucleus. NE-Necrosis. SLP-Swelling of Lamina Propria.

Discussion

The digestive system is varying in fish species to species. It is also depends upon habit, habitat and kind of food. The digestive system of the carnivorous *schilbe Mystus* having a caecal type stomach with a highly distensible caecum, which may be used as a reservoir ⁵. However, the carnivorous fishes with a predatory habit and irregular intake of large quantities of food possess a caecal type stomach¹. *Danio aequipinnatus* is also carnivorous fish. Then, the architecture of the stomach of *D. Aequipinnatus* fish is also caecal type. The wall of the stomach of *Danio aequipinnatus* consists of four layers: mucosa, sub-mucosa, and muscularis and serosa.

Pesticides cause histological, biochemical and physiological changes in fish species and influence their activities. Newtech cause damage not only to the absorptive area of the stomach but they also disturb the physiological processes and absorption of digested food. Similar results were reported by Shete and Patwari⁹, in *Macrones cavasius* using CuSO₄ as a pesticide. Takashi ¹¹, stated that pesticidal treatment induced several abnormalities stomach tissues; gastric mucosal ulcer action, lack of appetite. In the present study Necrosis, vacuolation in sub mucosal region, degeneration of columnar cells, rapturing of epithelium, destruction of goblet cells also observed. Similar results were recorded by Roma and Mishra⁷, in stomach of *Mystus tengara* using organophosphate as a pesticide. Vacuolation of muscularis layer and submucosa has been reported from different test cases⁸. Shete and Patwari⁹, recorded pyknotic nuclei, necrosis in gastric gland, and vacuolation in mucosal layer of *Macrones cavasius* exposed in sublethal concentration of CuSO₄. Sever vacuolation in the gastric epithelium and disruptions in the tubular gastric glands of the stomach were observed by Smaranya *et al.*,¹⁰ in *Channa punctatus* exposed to Fluoride.

Conclusion

The present investigation has revealed degenerative effects of the biopesticide newtech on the stomach of fresh water cyprinid *Danio aequipinnatus*. *Danio aequioinnatus* exposed to the sub-lethal concentration of the pesticide. Histopathological changes were Necrosis, vacuolation in sub mucosal region, degeneration of columnar cells, rapturing of epithelium, destruction of goblet cells, degeneration of serosa layer, swelling up of lamina propria, damage of mucus epithelial cells and mucus in the lumen of the stomach hypertrophied epithelial cells of the mucosa and gastric glands with pyknotic of nuclei and clear cytoplasm, completely rupture of gastric glands, separation of gastric gland cells from the basement membrane forming cluster like structure rupture of muscularis layer or all over the entire stomach tissue. Authors findings are also well in agreement with the findings of may previous investigators. Thus, it can be concluded that stomach of the

fresh water fish *Danio aequipinnatus* undergo severe histopathological alterations when exposed to the chronic concentration of the pesticide newtech.

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Refferences

- Carrasson M, Grau A, Dpazo L.R, and Crespo, S. "Histological Histochemical and Ultrastructural Study of the Digestive System of *Dentex dentex* (Pieces Sparidae). ." *Jounal of Histology and Histopathology.*, 2006: 21(6): 579-593.
- Cengiz El, Unlu and Balci K., "The Histopathological Effects of Thiodon on the Liver and Gut of Mosquito fish. *Gambusia affinis*." *Journal of Environmental Science and Health*, 2001: B.36 (1): 75-85.
- Ganeshwade R.M. "Histopathological Changes in the Gills of *Puntius ticto* (Ham) Under Dimethoate Toxicity." *The Bioscan*, 2012: 7(3):423-426.
- Murrey H.M., Wright G.M and Goff G.P.,. "A Comparative Histological and Histochemical Study of the Stomach From Three species of Pleuronectid, the Atlantic Halibut, *Hippoglossus hippoglossus*, the Yellowtail flounder, *Pleuronectes ferruginea* and the Water Flounder, *Pleuronectes americanus*." *Canadian Journal of Zoology.*, 1994: 72: 1999-1210.
- Naguib S.A.A, Shabaka H.A.El and Ashour F.,. "Comparative Histological and Ultrastructural Studies on the Stomach of *Schilbe mystus* and the Intestinal Swelling of *Labeo niloticus*." *Journal of American Science.*, 2011: 7(8):251-263.
- Rebolledo I.M and Vial J.D. "Fine Structure of the oxynticopeptic cell in the gastric glands of an Elasmobranch Species (*Heelurus chilensis*)." *Anatomical Record*, 1979: 193: 805-822.
- Roma Kumari and B.K.P. Mishra. "Effects of Pesticid on the Histology of Stomach and Liver of A Water Breathing Teleost, *Mystus tengara*." *Research Journal of Chemical and Environmental Sciences.*, 2015: 3(5): 32-36.
- Sharma R.R, Pandey, A.K. and Shukla, G.R.,. "Histopathological Alterations in Fish Induced by Pesticides Toxicity." *Journal of Aquaculture*, 2003: 2(1): 31-43.
- Shete P.S and Patwari J.R. "Acute Toxicity of CuSO₄ In The Fresh Water Fish: Histopathology Of The Stomach ."
- Trends in Life Science.*, 2012: 1(1): 36-37.
- Smaranya Haque, Sandipan Pal, Alope K Mukharjee, Apurba R Ghosh.,. Histopathological and Ultramicroscopic Changes Induced by Fluoride in Soft Tissue Organs of the Air-Brething Teleost, *Channa punctatus* (Bloch). Research Report Fluoride., New Zealand: The International Society for Fluoride Research Inc., 2012.
- Takashi Hibiva. "An Atlas of Fish Histology: Normal and Pathological Features Gustave Fischer Verlag, Stuttgart, New York." 1982: 74-85.
- Thorat S.R. "Histopathological Changes in the Intestine of Freshwater Fish Chaqna gachua Exposed to Tannery Effluent." *Journal of Pollution Resources.*, 2001: 20(2): 267-270.