Impact of heavy metal cadmium nitrate on histopathology of Intestine of fish Channa punctatus

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***Abstract*- This study was carried on fish channa punctatus to investigate the effect of lethal concentration of cadmium nitrate on histopathological study of intestine compare to control one.**
96 hr. LC_{50} for cadmium nitrate is 0.57 ppm. Which is toxic to fish intestine. Degenerative effect is evident in the mucosal lining and villi of the intestine. The villi is to become flattened, sloughing off the mucosal lining, hypertrophy of epithelial cells swelling, fusion of villi due to extensive hypertrophy of epithelial cells swelling, fusion of villi due to extensive hypertrophies leading to rupture of villi at the tip and cracked clay appearance, degeneration of circular musclelayer swelling in lamina propria hypertrophied epithelial cell and swelling of laminal propria were noticed

***Key words*- Pollution, cadmium nitrate, Histopathology, Intestine, channa punctatus.**

Introduction:

Environmental pollution caused by the development of industries, urbanization and informal settlements does though threaten many freshwater ecosystems. Environmental pollution causes a decrease in water quality and after ward affects all living organisms in that system. It is necessary identify and manage these pollution sources, but also to monitor their effects on the aquatic ecosystems. Fish is one of the sensitive to changes in their surrounding environment including an increase in pollution. Fish health may thus reflect and give a good indication of the health status of a specific aquatic ecosystem. Early toxic effects of pollution may evident on cellular or tissue level before significant changes can be identified in fish behavior or external external appears. Histological analysis is a one of the technique to assess the sensitive parameter and is crucial in determining cellular changes that may occur in target organs, such as the gills, liver and gonads (Datta, 1996). Some of the heavy metals are necessary for specific bodily functions due to their nutritional value but the highest concentrations of these metals cause toxic effect within an organism. These toxic concentrations of metals may vary to specific for the specific species of aquatic organism. This variation has been seen in mammals but also seen in various fish species. Meyers Hendricks (1982) studied on the heavy metals substances are harmful to fish at very law concentration. Heavy metal pollution in aquatic ecosystems, especially river systems, is a major environmental concern It is therefore necessary to investigate not only the possible organism and population response to heavy metal pollution, but also the underlying causes of the specific response, for example histological alterations or damage caused by the specific exposure. Fish populations will either adapt to environmental changes, or may die. Histological analysis is not always a simple task, even in mammalian pathology, which has much more extensive database if compared to that of fish pathology (Hinton and lauren,1990).

MATERIAL AND METHOD :

The selected mode animals the freshwater fish channa punctatus were collected from yeldari dam parbhani district. After collection the fishes were acclimatized in the laboratory condition of room temperature for 2-3 days. The active acclimatized fishes of approximately same size were size were selected for experiment.

Before starting the experiment these fishes were divided into two groups one group of fishes was maintained as control while the second groups was exposed to chronic does of cadmium nitrate 96 hrs. LC_{50} 0.57ppm.

Processing of tissues for histopathological studies.

The tissue removed from the test fish washed in 0.90% saline saline solution for two times for remove blood or debris attached on external surface. The tissues were then cut into small pieces of approximately 3-5 mm.

Fixation of Tissues :-

The aim of fixation is the preservation of cells and tissue, existing during life, prevention of autolysis, loss of easily diffusible substances by appropriate coagulation. Tissues were fixed in Bouine's fluid. The tissue was kept in fixative for 6-24 hrs. (Drury and wallington, 1980)

Post fixation process :

After fixation of tissue washed three times in clean cold water to remove yellow colour.

Dehydration :-

Ascending grades of aquous ethyl alcohol beginning with 30%, 50%, 70%, 90% and absolute alcohol. Then clean with xylene for 2 min. and transferred in xylene + melted paraffin mixture (1:1) for one hour. Tissues were transferred in to melted paraffin at room temperature till solidification (Drury and wallington, 1980)

Embedding :-

To lubricate a thin layer of glycerol was placed in cavity. Melted wax was poured and allowed to stand for sometimes with warm forceps tissues were taken and put in the middle cavity. Which then placed in cold water and blocks were removed. Surplus wax was cut from sides.

Section cutting :

5 um thicknesses of sections were selected in the form of ribbons of 10-15 cm in length.

Mounting :

A mixture of egg albumin and glycerol was smeared very thinly on slide before mounting. Section was gently lowered onto the water surface (5.10^0C) sections were flattered by gentle heat (Drury wallingtion,1980) De-waxing was carried out by giving two washes in xylene for 5 min.

Hydration :-

Sections were run through down grading of alcohol i.e 90%, 70%, 50%, 30%, alcohol for 2-5 min.

Staining :-

Stained with Haemotoxyline for 15-20 min. Then washed slide under running tap water for 2-3 min. Then quick dip of 0.5 -1% Hcl in 70% alcohol for a few seconds. Then stained the slide in 1% aqueous Eosin for 1-3 min. slide was dipped in 90% and 100% alcohol for a few minutes.

Mounting :-

For drying and preservation slides were kept at room temp. for 3-4 days.

Microphotographs of slides:-

After 4 days, mounting slides were used for microphotographs, done by binocular microscope (Labomed LX-400) with attached camera ivu 5100 labomed.

Result

Histopathology of intestine: (Control)

Intestine is the important organ of digestive system of fishes. The functional importance of intestine is absorption. Maximum absorption of digested food products takes place in intestine. The control structure shows columnar epithelium; circular muscle layer, lamina propria, Serosa, Submucosa and longitudinal muscle layer were observed (Fig.no1A)

Histopathology of intestine: (Experimental)

In the present investigation intestine shows normal structure in control fish but in exposed fish degenerative effect is marked in the mucosal lining and villi of the intestine. The villi is to become flattened, sloughing off the mucosal lining, hypertrophy of epithelial cells swelling, fusion of villi due to extensive hypertrophy of epithelial cells swelling, fusion of villi due to extensive hypertrophies leading to rupture of villi at the tip and cracked clay appearance, degeneration of circular musclelayer swelling in lamina propria hypertrophied epithelial cell and swelling of laminal propria were noticed (fig. no. 2A)

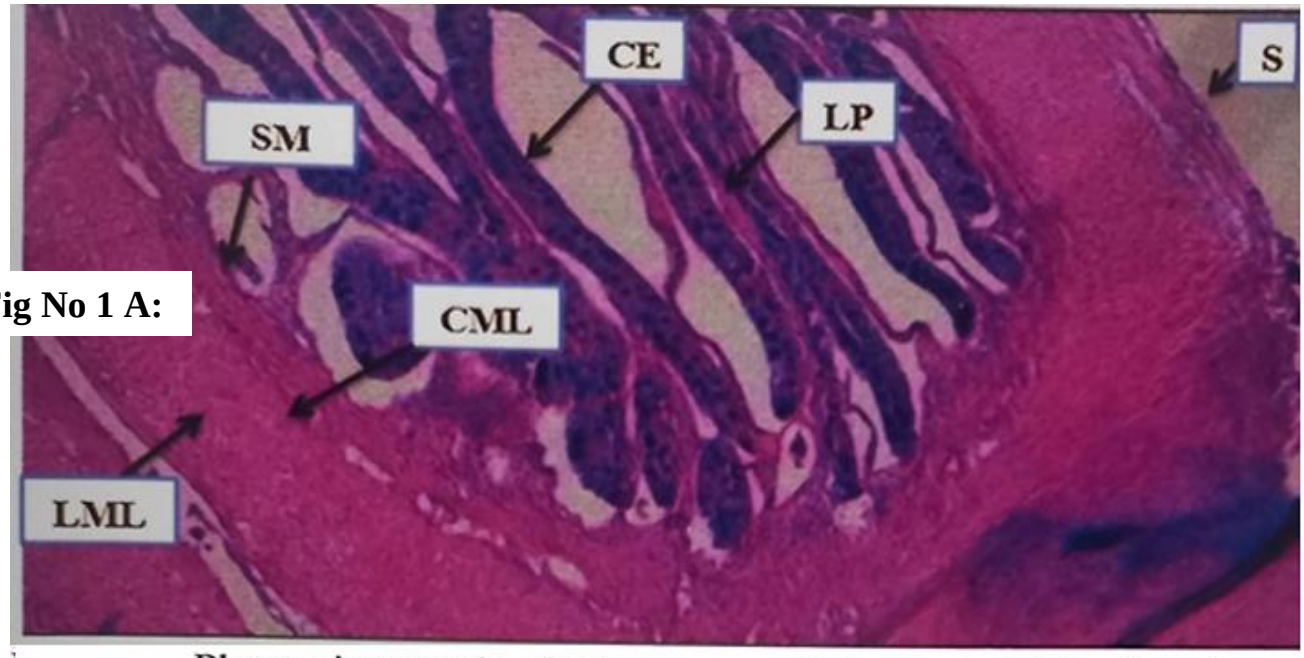
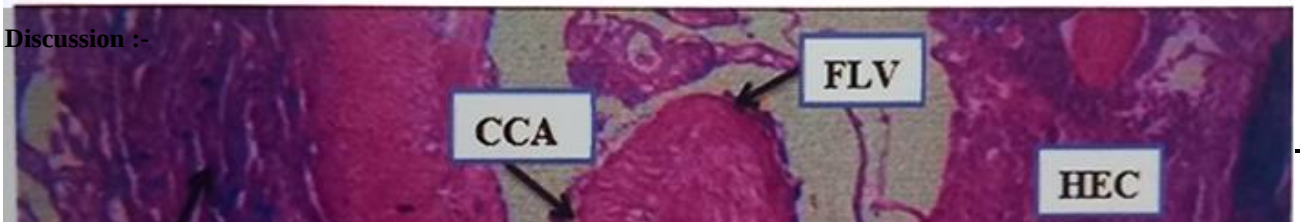


Fig No 1 A:

Fig No 2 A: Photomicrograph of *Channa punctatus* control intestine shows Columnar epithelium (CE), Circular muscle Layer (CML), Lamina propria (LP), Serosa (S), Submucosa (SM) and Longitudinal muscle layer (LML) Control Intestine H/E- 100X.

Discussion :-



Chhaya Bhatnagar, et.al. (2007) studied on fluoride-induced histopathological changes in intestine of fresh water teleost, Labeo rohita and reported that degenerative effect is evident in the mucosal lining and villi of the intestine. The villi tend to become flattened, and there is sloughing off of the mucosal lining.

Juan Ortiz, et.al. (2003) worked on histopathological changes induced by lindane (y YCH) in organs of fishes and reported in exposed group a degenerative effect is evident in the mucosal lining and villi of the intestine. The villi tend to become flattened, and there is sloughing off of the mucosal lining. Flattening of microvilli and a cracked clay appearance of the tissue are likewise apparent. Shanta satyanarayan, et.al. (2012) studied on histopathological changes due to some chlorinated hydrocarbon pesticides to Cyprinus carpio and reported that the intestine the flattening of intestinal folds, fusion with each other, shrinkage of cells and acute epithelial necrosis, more flattening of intestinal folds. In 30 days exposed tissue the intestinal folds were completely flattened thereby reducing the surface area, necrosis was also very defined, vacuolation and acute necrosis were observed.

Muley, et.al. (1996) worked on the intestine of channa punctatus after the exposure to carbofuran. They reported that degenerative changes and rupture in tip of villi, loss of structural integrity of mucosal folds and degeneration and necrosis of submucosa are found in intestine while working on endosulfan toxicity in the freshwater fish Tilapia mossambica. Sastry and sharma (1979) report on vacuolization in submucosa and circular muscles and dilation of columnar and goblet cells of mucosal folds while working on toxic effects of endrin on liver and kidney of a freshwater fish.

Ghanbahadur and Ghanbahadur (2012) studied on toxicity of endosulfanon Rasbora daniconius and reported that the destruction of mucosa and particularly the columnar epithelial cells in the intestine. Damage of brush border on the luminal surface of the interior. Furthermore, the disruption of blood vessels in the submucosa might impair its ability of absorption (Ghosh, 1990) Velmurugan, et.al. (2007) when exposed cirrhinus mirgala to sub lethal concentration (0.3 ppb and 0.6 ppb) pf lambda cyhalothrin (a synthetic pyrethroid pesticide) observed intestinal lesions, infiltration of eosinophils into lamina porpia and atrophy of epithelial cells. Flattening and cracked clay appearance was observed by Bhatnagar, et.al. (2007) after chronic exposure for 30, 60, 90 and 120 days of fingerlings of labeo rehita to 15,g NaF/L.

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