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CADMIUM CHLORIDE INDUCED HISTOPATHOLOGICAL CHANGES IN THE GILL, INTESTINE, LIVER AND KIDNEY OF FRESHWATER FISH, RASBORA–DANICONIUS.

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Short Profile

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ABSTRACT:

Industrial evolution and growing population has created a main problem of heavy metal pollution to aquatic life because of their toxicity resistance and tendency to accumulate organism and food chain (Weis and Weis 1977). The present study was undertaken **to determine the histopathological changes in gills and intestine of Rasbora-daniconius due to cadmium toxicity**. Fish Rasbora-daniconius were exposed to 110ppm cadmium chloride (Cdcl2 2 ½ H2O) (Lc50 for 24h) for 24 hour. It was observed that, **in gills epithelial walls of each secondary lamella were damaged the secondary lamella were ruptured pillar cells were displaced with vacuolation intralamellar distance distributed marked degenerative changes were noticed in pillar cells, blood capilllaris epithial cells and blood cells. In intestine the columnar epithelium lining was destructed the absorptive cells and the mucosa secreting cells were damaged. The four structured border was ruptured the outermost serosa innermost mucosa and muscularis and sub mucosal were damaged. The overlying mucosa was lost deep folds were apparent gaps between mucosal and sub mucosal layers and intracellular vacuolar in the sub mucosal were apparent**

KEYWORDS

Fish, Cadmium toxicity, gill, intestine, liver and kidney.

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INTRODUCTION :

Heavy metals contaminats arising out of industrial effluents enter into ecosystem through aquatic reserviour and they cause adverse effects on aquates productivity and living resources.

Heavy metals salt construct a very serious type of pollution to aquatic biota because they are stable compounds and are not readily removed by oxidation, precipitation or other means and effect the activity of animals (costa 1965)

Dhanapakiam et.al. (1998) studied histopathological changes in gills of Channa punctatus in canvay river water. Banergee and Bhattacharya (1994) studied histopathology of kidney of Channa punctatus exposed to chronic non-lethal level of elsan, mercury and ammonia.

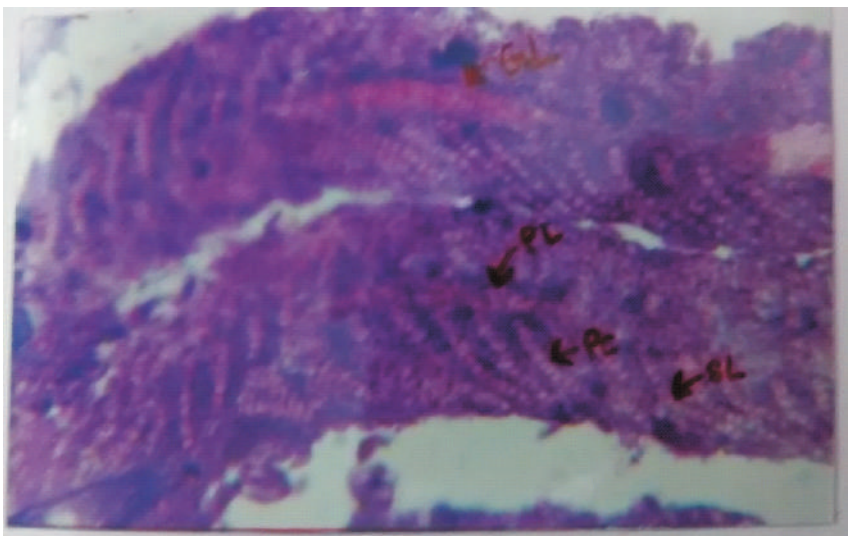
Vallflery and Kerchoas (2000).Studied the effect of cadmium on the reproductive system of the land snail Helix aspersa.

A Ghosh and Chakrobarti (1993) reported histopathological and histochemical changes in the liver pancreas and kidney of the freshwater fish Heteropneustes-fossilis. Exposed to cadmium.

Hence, in the present study, the effect of cadmium chloride is studied on histological changes in gills and intestine liver & kidney of the fish Rasbora- daniconius.

MATERIAL & METHOD:

The freshwater fishes, Rasbora- daniconius were collected from Godavari River at Paithan, 55km south of Aurangabad. They were kept in glass aquaria with sufficient quantity of tap water for 2-3 days to acclimatize them to the laboratory conditions. Healthy fishes were **subjected to cadmium chloride treatment**. Fishes having approximately equal size (7to 9cm body length) and weight (5 to 7g) were selected for experimentation. The **experimental fishes were exposed to 110 ppm cadmium chloride (Cdcl2 2 ½ H2O) Lc50 for 24h**. Aeration and feeding was discontinued for the duration of experiment. After the exposure period, the fishes were **carefully and quickly dissected**. Tissues like gills, intestine, liver, and kidney were taken out washed and fixed in aqueous Bouins fluid for 24h. After fixation the tissues were passed through 30% to 100% of alcohol for dehydration and cleared in xylol. They were embedded in paraffin wax (M.P. 500-600C) and series of sections were cut at 7-8µm. the sections of gills and intestine were stained in Delafield's Haematoxylin and counter stained with eosin Y (Bancraft and Steven, 1982), **Damage to the treated tissues of gills and intestine were recorded by comparing the data obtain each concentration with that of control**.



RESULT:

Fig. 1

•L.S. of the gills of the normal freshwater fish, Rasbora –daniconius, stained with haematoxylin and eosin x 200

Note the lamellae are regularly spaced, the row of pillar cells are clearly seen. Each secondary lamella is closely adhering the respiratory epithelium.

(Scale 1cm=10 µm)
PL- Primary lamellae
SL- Secondary lamellae
Gl- Gill ray
Pc- Pillar cell

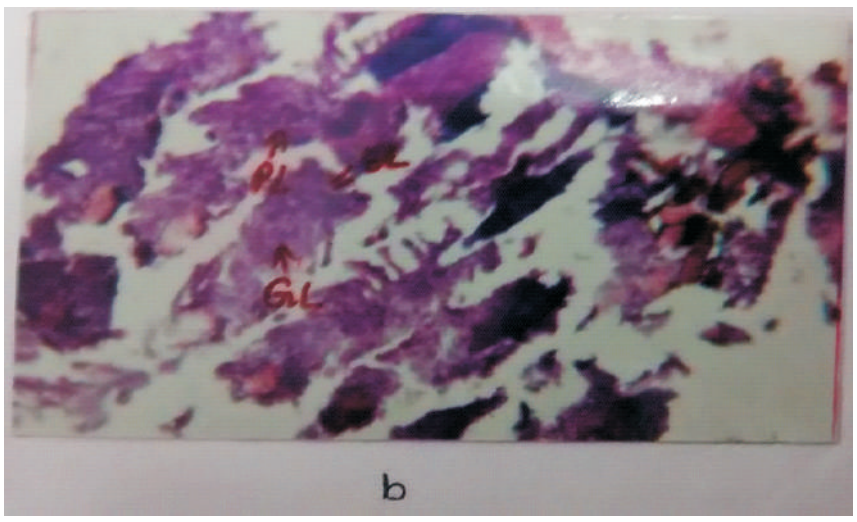


Fig 1b

•L.S. of the gills of Rasbora- daniconius showing the effect of cadmium chloride after exposing to 110 ppm for 24h.

Note the damaged epithelial walls of secondary lamella, and the displacement of pillar cells. The gill filament and gill rays showed distortion. The interlamellar spaces were filled with cellular debris and mucous. Stained with haematoxylin and eosine x 100

(Scale: 1 cm=10 µm)
Pc- Pillar cell
Gl- Gill ray
SL- Secondary lamellae

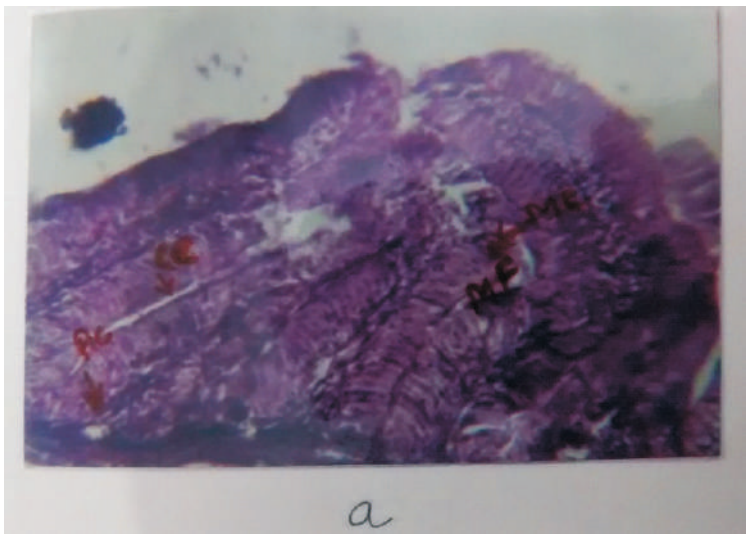


Fig 2a

T.S. of the intestine of normal (control) fish, Rasbora daniconius stained with q stained with haematoxylin and eosin x 100

Note simple columnar epithelium lining consisting of absorptive cells and the mucus secreting cells. The muscularis of the intestine is thin and consist of outer most serosa, innermost mucosa and muscularis submucosa sandwitched between the two. The submucosa with overlying mucosa was thrown into many deep folds (scale: 1cm=10 µm)
MC- Mucous secreting cells
MF- Mucosal folds
AC- Absorptive cells
CC- Columnar epithelial cells

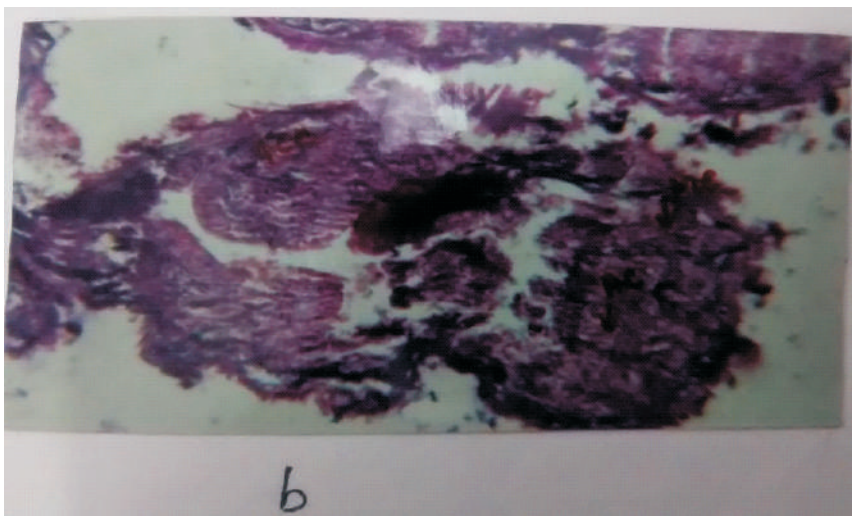


Fig 2b

T.S. of the intestine of fish Rasbora daniconius showing the effect of cadmium chloride exposed to 110ppm for 24 h.)

Note simple columnar epithelium lining was destructed the absorptive and mucus secreting cells were damaged. The free striated cells were damaged. The granular cells present in the mucosa were degenerated. Gaps between mucosal and submucosal layers and intercellular.

Vacuolation in the mucosa were developed. Stained with haematoxylin and eosine x 100
(Scale: 1cm= 10 µm)
MC- Mucous secreting cells
AC- Absorptive cells
CC- Columnar epithelial cells

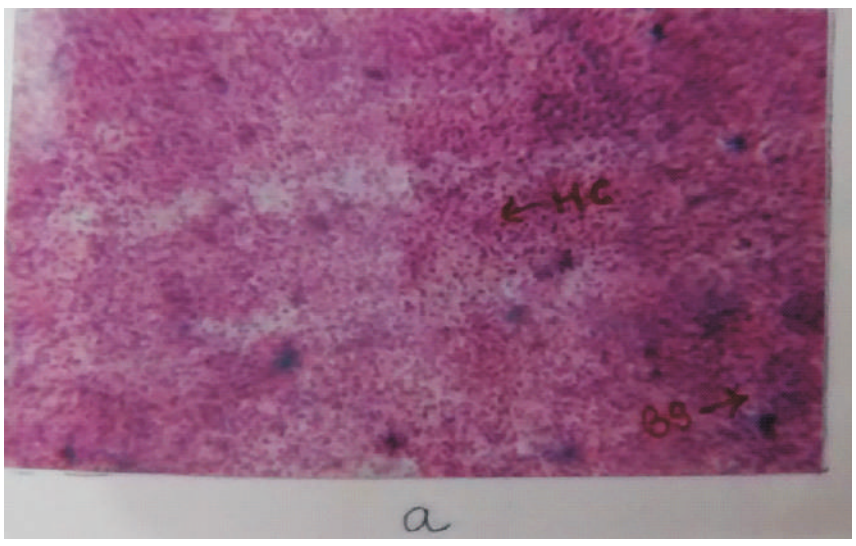
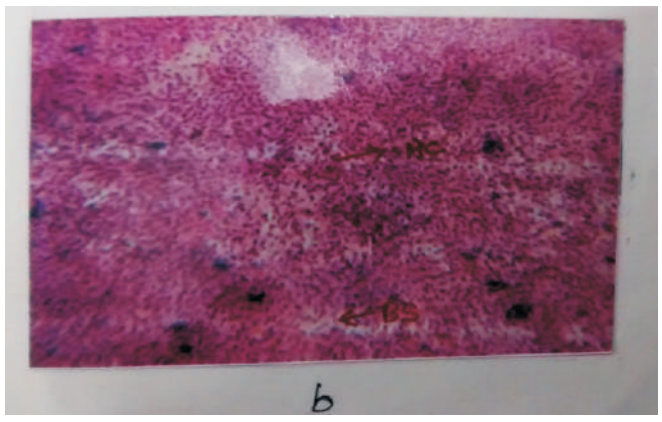


Fig. 3a

T.S. of the liver of normal (control) fish. Rasbora daniconius stained with haematoxylin and eosine x 100.

Generally round, polygonal hepatic cells containing large characteristics spherical nuclei. Blood sinusoids between the hapatocytes can also be seen.

(Scale: 1cm=10µm)
HC- Hepatic cells

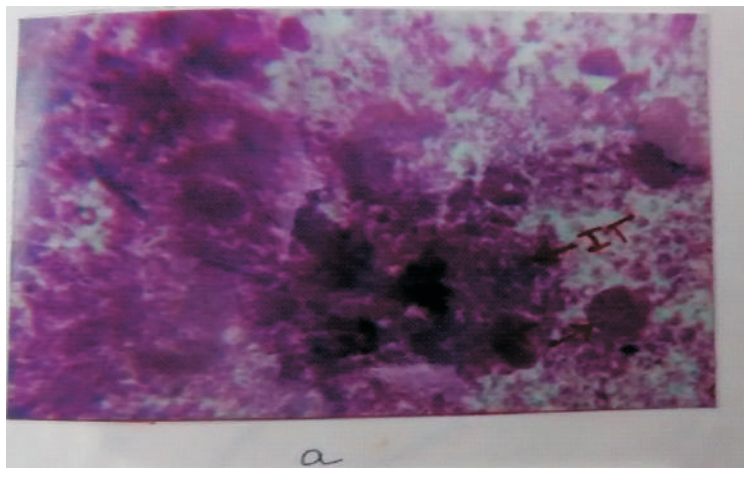


BS-Blood sinusoids

Fig.3b

T.S. of the liver of fish, Rasbora daniconius showing the effect of cadmium chloride exposed to 110ppm for 24h. Vacuolation in the cytoplasm and cloudy swelling of the nucleus with large vacuoles in hepatocytes were observed. The swollen nuclei and cytoplasmic vacuolation, gaps between hepatocyte and loss of cell boundaries were noticed. Stained with haematoxylin and eosine x100

(Scale: 1cm=10µm)
SHC= Swollen Hapatic cells



SN= Swollen nuclei
Fig. 4a

T.S. of the kidney of normal (control) fish, Rasbora daniconius, stained with haematoxylin and eosine x 200.

The uriniferous tubules lined by cuboidal epithelium and clusters of interstitial tissues can be seen

(Scale: 1cm=10µm)

UT- Uriniferous tubule

IT- Interstitial tissue

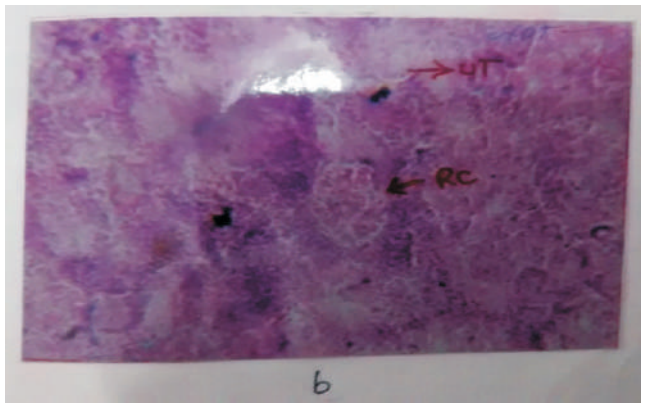


Fig. 4b

T.S. of the kidney of fish, Rasbora daniconius showing the effect of cadmium chloride exposed to 110ppm for 24h. The cells of some uriniferous tubules show dilatation and cytoplasmic vacuolation. The tubule diameters were found increased with a decrease in the haemopoietic tissue. Damage to cells and renal capsule was also noticed. Stained with haematoxylin and eosine x200.

(Scale: 1cm=10µm)

RC-Renal capsule

UT- Uriniferous tubules

DISCUSSION:

The study of microanatomy of the specific tissue can constitute an important diagnostic tool to study the histological effect by pollutants. Changes in the histological and histochemical structure are mainly directed to study the effect of pollutants on the structural component of cell. The potential toxic pollutant induce morphological, behavioural and physiological changes in the vital organs such as respiratory, nervous, osmoregulatory, reproductive etc. (Fingerman, 1982).

The gills of fishes are vital respiratory structures which are important in the homeostasis of the milieu interior. Gills are also responsible for regulating the exchange of salt and water and play a role in the excretion of nitrogenous waste products. Normally the gills have arches composed of rows of filaments having distinct lamellae projecting from each side.

STRUCTURE OF CONTROL (NORMAL) GILLS

In the fish Rasbora daniconius the normal gill consists of a row of four arched gill bars. Each gill bar carries along its length on either side, two rows of elongated flat gill filament the primary lamellae.

Each filament is supposed by a cartilaginous gill ray which acts as a mechanical support. Each gill arch consisted of a primary filament composed of connective tissue and a number of secondary gill filaments at right angles to it and spaced regularly. On the upper and lower surfaces of each filament are series of thin parallel folds, the secondary lamellae which from the respiratory surfaces.

The epithelial walls of each secondary lamella are supported by core of pillar cells. A blood space is present within the lamella which communicates with the small afferent and efferent blood vessels in the filament. (fig.1a).The epithelial wall of the secondary lamella is single layered. The epithelium of the primary lamella is multilayered and it possesses numerous mucous cells. Mucous cells do not occur in the secondary lamellae. The pillar cell system was covered by a thin flat respiratory epithelial cell layer.

EFFECT OF CADMIUM CHLORIDE ON GILLS OF RASBORA DANICONIUS.

The result of the present investigation showed several striking changes in the gill architecture as a consequence of cadmium chloride toxicity (Fig.1b). The gills Rasbora- daniconius exposed to 110ppm of cadmium chloride for 24h showed disintegration of gill epithelium vacuolation of gill lamellae displacement of pillar cells and interlamallar spaces, and marked degenerative changes in the blood cells, epithelial cells, blood capillaries and pillar cells. Gupta et. al. (1995) observed similar changes in the gills of Rasbora- daniconius due to mercury poisoning whereas Srivastava and Gupta (2000) observed similar histopathological alteration in the gills of another fish, Channa punctatus due to zinc toxicity. S. Das and A. Gupta (2012) studied effect of cadmium chloride on oxygen consumption and gill morphology of Indian flying barb, Esomus danricus.

Since, gills are in direct contact with the toxic medium they are organs which are the first affected by the lethal and sub lethal toxic concentration of many substances (Bilinki and Jones 1978., Hinton et. al. 1973; Gupta and Rajbanshi, 1979; Shrivastava and Shrivastava 1979)

The damage due to cadmium chloride intoxication in the gill results in a fall in the capacity of the gills for gaseous exchange. Consequently the rate of oxygen consumption was markedly lowered in the experimental fish Rasbora daniconius

The lowered level of oxygen consumption is obviously due to suffocation caused by the thick film of mucous on the surface of the gills.

STRUCTURE OF NORMAL (CONTROL) INTESTINE OF RASBORA DANICONIUS

Intestine is lined by a simple columnar epithelium consisting of absorptive cells and the mucous secreting cells. The former possess a free striated border. The mucus secreting cells are fewer in number in the anterior part of the intestine, in the middle region but increase considerably in the posterior part. A few granular cells, basophilic or acidophilic in nature, are also consists of outer most serosa, innermost mucosa and muscularis, submucosa sandwitched between the two. The sub mucosa with overlying mucosa was thrown into many deep folds. Also presence of microvilli along the free surface of the columnar epithelial cells of the intestine was observed and these cells are mainly concerned with absorption of digested food. (Sinha, 1994) (fig.2a)

EFFECT OF CADMIUM CHLORIDE ON THE INTESTINE.

The intestine of Rasbora- daniconius exposed to cadmium chloride for 24h showed changes in its structure. Mucosal disruption at the tips of folds and appearance of gaps between mucosal and sub mucosal layer and intracellular vacuolation in the submucosa were noticed the columnar epithelium lining was destructed. The absorptive cells and the mucous secreting cells were damaged. The free striated border was ruptured. The granular cells present in the mucosa were degenerated. The outer most serosa, innermost mucosa and muscularis and submucosa were damaged. The over lining mucosa which is thrown into many deep folds destructed and cell membrane of the columnar epithelial cells located in the tips of the intestinal villi were degenerated and the most of the intestinal villi showed cloudy appearance due to in distinct cells membrane (in Figure 2b). Gardner and Yevich 1970 have given an account of time dependent graded changes in the intestine of Fundulus heteroclitus when exposed to cadmium. Many workers have noted histopathological damage in the intestine in fishes exposed to various toxic substances (Ellis, 1937.Gardner and Yevich 1970, Srivastava et. al.1979. Srivastava and srivastava 1979). Vacuolation, necrosis, pynotic nuclei of epithelial cell, shortening and rupturing of villi and dead cell debris in lumen, congested blood capillaries and rupture of serosa layer were observed in the intestine of Channa punctatus when exposed to phenyl mercuric acetate. (Karuppaswami, 2000). Patil et. al. 2000 reported on histopathological changes in the lever of Nemacheilus botia due to exposure to dimecron.

STRUCTURE OF NORMAL LIVER OF RASBORA DANICONIUS

The normal liver of R. daniconius consists of parenchymal cells (hepatic cells) or hepatocytes and lattic fibres which support the hepatic cells. The liver is covered by serous membrane and the connective tissue from it expend inward into the parenchyma. The portal vein carries blood from stomach and intestine to liver and branches of and eventually divide into relatively wide blood capillaries known as sinusoids. Hepatic cells forming cord like structure known as hepatic cell cords in each of which is located a bile canaliculi. The hepatic cells are generally round, polygonal and contain spherical nucleus with a nucleolus and other inclusions like mitochondria, golgi apparatus, endoplasmic reticulum and others in the cytoplasm which appears at many vacuolar structure in hepatic cells.

Hepatic cells secrete bile which is poured into duodenum through bile duct and hepatic duct. Metabolism of food items, its storage and detoxification are also important functions of liver. The harmful substances reach liver through blood and hence liver is susceptible to a number of toxic and metabolic disturbances. (Fig.3a)

EFFECT OF CADMIUM CHLORIDE ON LIVER.

The fish exposed to 110 ppm for 24 h showed vacuolation in the cytoplasm. Cloudy swelling of the nucleus with large vacuoles was observed in hepatocytes. Cell showed loss of cell membrane and the cells were disarrayed. The swollen nuclei and cytoplasmic vacuolation and gaps between hepatocytes and loss of cell boundaries. The nuclei become pycnotic and seems shifted to the side of the cells, necrosis is prominent. (fig 3b) .In N. Suresh (2008), observed effect of cadmium chloride on

liver, spleen and kidney melano macrophage centres in Tilapia mossambica

STRUCTURE OF NORMAL (CONTROL) KIDNEY OF RASBORA DANICONIUS

The normal histological structure of kidney of Rasbora daniconius show large number of uriniferous tubules, each with a renal capsule a short neck, proximal and distal convoluted tubule and collecting duct. Study of the uriniferous tubules revealed that the cells of different size and shapes lined the tubule at different regions. These tubules were seen to be surrounded by interstitial haemopoeitic tissue. In the head of kidney were found a few oval or columnar inter renal cords made up of two rows of cuboidal cells with vesicular nuclei. In close proximation of these interrenal cords were seen aggregated chormaffin cells forming islets of phaeochromic tissue of various sizes. The clusters of haemopoeitic tissue components, the interstitial cells filled up the spaces between the tubules. (Fig 4a) Effect of cadmium chloride on kidney.

The fish exposed to 110ppm for 24h indicated that, cells of some tubules showed dilatation and cytoplasmic vacuolation with some increase in the tubule diameter and decrease in haemopoietic tissue. The renal capsule was also distructed. Damage to cell, size and shapes was observed. Enlarged nuclei were distinctly seen with hyperchromatic and extensive damage to renal tubules. The cuboidal cells with vesicular nuclei were damaged. The cells lining the lumen intermingle and rupture the tubules, some damage, to glomeruli and severe damage to ducts. The interrenal cells showed hypertrophy. (fig.4b) Nusrul Amin et.al. in (2013) study effect of cadmium chloride on the histoarchitecture of kidney of a freshwater catfish, Channa punctatus.Hajrudin Besirovic et. al. (2010) observed histopathological effects of chronic exposure to cadmium and zinc on kidneys and gills of Brown Trout, (Salmo tutta m. fario)

CONCLUSION:

The histopathology is a most common tool for determining the deleterious effect of toxic substances on the treated animals. In the present investigation, an attempt has been made to evaluate the intensity of the damage done to different organs of fish sublethial to its lethal concentration of cadmium chloride.

•In the fish, Rasbora daniconius the gills showed that, the epithelial walls of secondary lamella were damaged and ruptured. The pillar cells were displaced with vacuolation in some of them and separation of respiratory epithelium from the pillar cells at certain places was observed. Marked degenerative changes were noticed in pillar cells, blood capillaries, epithelial cells and blood cells.

•The intestine of the fish, Rasbora daniconius showed that, the mucus secreting cells were damaged. The free striated border, outermost serosa, innermost mucosa and muscularis and submucosa were damaged. The intracellular vacuolation in the submucosa were apparent. The cell membrane of the columnar epithelial cells located at the tips of the intestinal villi were degenerated and most of the intestinal villi showed cloudy appearance with indistinct cell membrane.

•The liver of the fish, Rasbora daniconius showed vacuolation in the cytoplasm. Cloudy swelling of the nucleus with large vacuoles was observed in hepatocytes. Cell showed loss of cell membrane and the cells were disarrayed. The swollen nuclei, cytoplasmic vacuolation, gap between hepatocytes and loss

of cell boundaries were observed. The nuclei became pycnotic and shifted to the side of the cells.

•The kidney of the fish, Rasbora daniconius showed dilatation and cytoplasmic vacuolation in some tubules, decrease in haemopoietic tissue. Renal cell size and shape were damaged along with distruction of renal capsule. Enlarged nuclei were distinctly seen with extensive damage to renal tubules. The cuboidal cells with vesicular nuclei were damaged. The cell lining and the lumen intermingle and rupture the tubules. The glomeruli and ducts were also damaged.

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