Architectural changes in kidney and liver of *Channa punctatus* when exposed to mercury

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Abstract: In the present investigation live specimens of *Channa punctatus* were collected, after acclimatization they were chronically exposed to sublethal concentrations of mercuric chloride for 30 days. As compared to the control fish, Mercury treated fish kidney & liver showed marked histopathological changes. The effect was dose and duration dependents. After 30 days exposure, marked shrinkage of glomerular tuft at all the concentrations were noticed in kidney. Further the effect became so severe that visceral epithelium got ruptured, glomerulus got evacuated and necrosis of kidney tubule was seen. Liver histology showed hepatic congestion and distinct cell boundaries were found to be destroyed. Liver also showed increases in the size of hepatic sinusoids.

Index terms- Mercury, LC_{50}, *Channa punctatus*, Kidney, Liver

I. INTRODUCTION

For the last few decades water quality deteriorates because of addition of pollutants from commercial properties, industrial and domestic waste and pollutants of varied origins, where these pollutants are found in their untreated form (Asano et al. 2007). As a result of this in India 70% of the available water is polluted, out of which 84-92% is polluted by sewage pollution and 8-16% by industrial pollution (Khapekar et al, 2008). Impact on fish scales as indicators of wastewater toxicity from international water channel Tung Dhab drain has been reported (Kaur and Dua, 2012).

Increase in the concentration of heavy metals (Cd, Cu, Fe, Ni, Mn, Zn, Pb and Hg) has been reported in water of Vasai Creek, Maharastra (Lokhande and Kelker, 1999). Heavy metals salts constitute a serious type of pollution in fresh water and being stable compounds, they are not readily removed by oxidation, precipitation or other processes and affect the activity of recipient organisms (Nammalwar,1985).

Mercury, one of the heavy metal once released it persists in the environment. It has been extensively used in industries like pesticides, electroplating, medicines and battery manufacturing (Seiler and Sigel, 1988). Effluents from these sources are ultimately dumped to aquatic ecosystem, where they harm non-target flora and fauna such as fish.

The present study was to study the impact of mercury on the kidney and liver architectural changes in *Channa punctatus* a fresh water fish.

II. RESEARCH METHODOLOGY

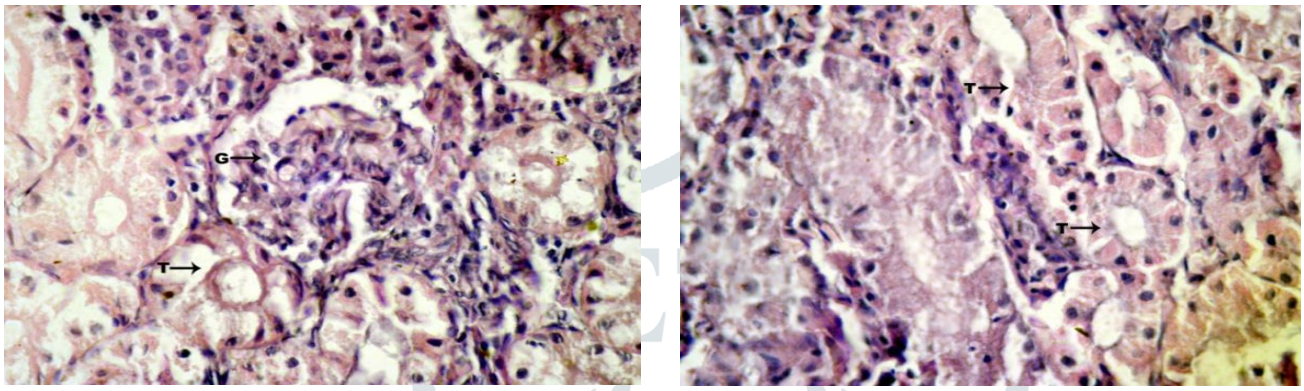
For the present studies live specimens of *Channa punctatus* were collected. They were given bath in 0.1% $KMnO_4$ for 2-3 minutes. The fishes were acclimatized for 7 days under laboratory conditions. Mercury in the form of mercuric chloride was used for present investigations. The salt is selected because of its uses in industries reported toxicity and water solubility.

LC_{50} value for present study was calculated by Probit analysis as suggested by Finney (1980). Based on the Probit analysis technique, 96h LC_{50} value was found to be 1.21 mg/L by graphical interpolation and arithmetic methods. A stock solution of 1 g/L was prepared in normal tap water. From the stock solution measured aliquots of this was added to each experimental tanks so as to bring the mercuric chloride concentrations to required levels i.e. 0.08 mg/L, 0.10 mg/L, 0.25 mg/L, 0.40 mg/L and 0.55 mg/L. The fishes were exposed to these concentrations for 30 days.

III. RESULTS AND DISCUSSION

According to the classification of the teleostean kidney as given by Ogawa (1961), *Channa punctatus* freshwater teleosts, bears type II kidney. It is divided into two portions, an anterior head kidney composed of hematopoetic, lymphoid and endocrine tissue and a posterior trunk kidney composed of numerous nephrons surrounded by interstitial lymphoid tissue.

General Structure of Kidney The basic unit of kidney is a nephron that in C*hanna punctatus* consists of renal corpuscle and renal tubule. Renal corpuscle is having a well-developed vascularized glomerulus (Fig. 1 a). The renal tubule consists of initial proximal segment (Proximal I) with prominent brush border, second proximal segment (Proximal II) with numerous mitochondria but less developed brush borded (Fig 1 b).

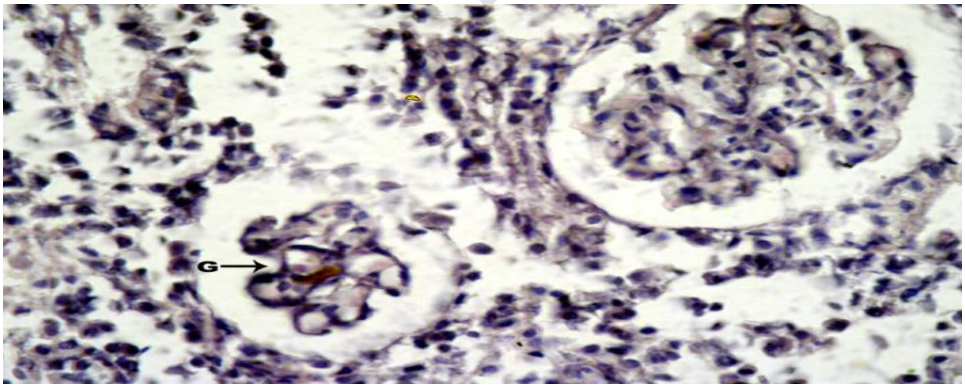


(a) (b)

Fig. 1: Photomicrographs of fish kidney (control), showing (a) glomerulus with parietal and visceral epithelium and (b) kidney tubules. G (Glomerulus)

Histopathological examination of fish kidney is an authentic method of determining impacts of contaminants in the aquatic environment (Cuppage and Tate, 1967 Tarzwell, 1971; Brown *et al.* 1977); during the present study, response of a teleostean (*Channa punctatus*) kidney to the sublethal concentration of Mercury has been studied.

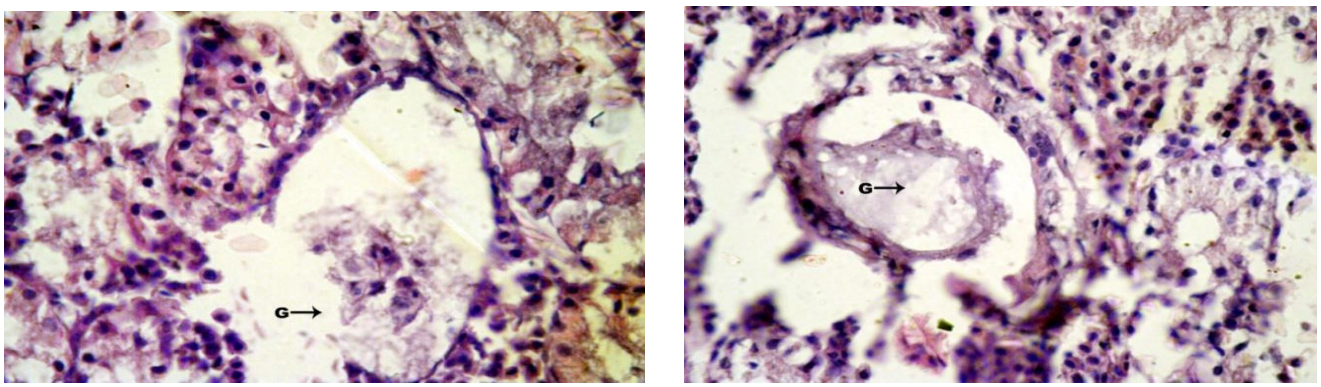
As compared to the control fish, Mercury treated fish kidney showed marked histopathological changes. The alterations detected initially were the widening of space between the capsule and the glomerular capillary network. on exposure period for 30 days more marked shrinkage of glomerular tuft at lower concentration (0.080mg/L Fig. 2a) was noticed. Further the effect became so severe that visceral epithelium also got ruptured and glomerulus got evacuated (3a and b 4a and b) at rest of the concentrations. Mercury also induced necrosis of kidney tubule of the fish (Fig. 5a and b).



(a)

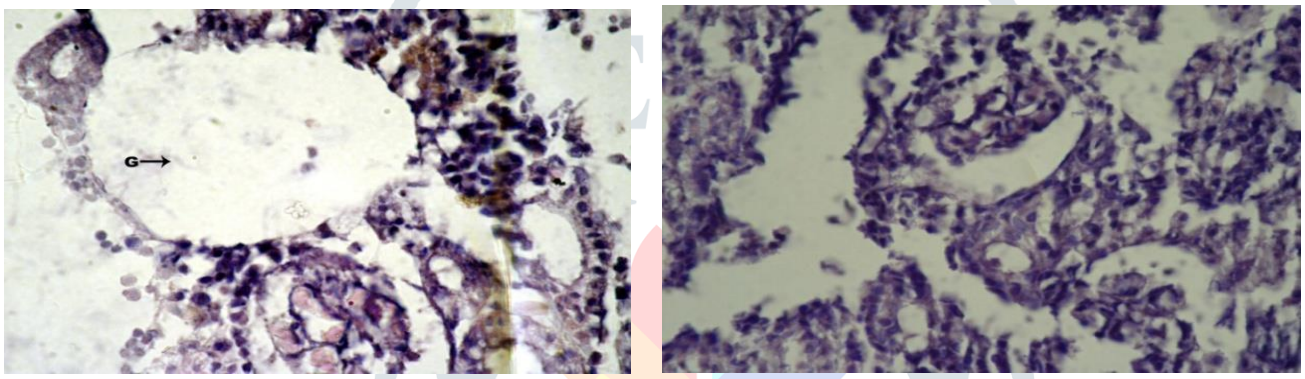
Fig. 2: Photomicrographs of fish kidney (Mercury treated) showing shrunken glomerulus

(a-0.080 mg/L, 30days) G (Glomerulus)



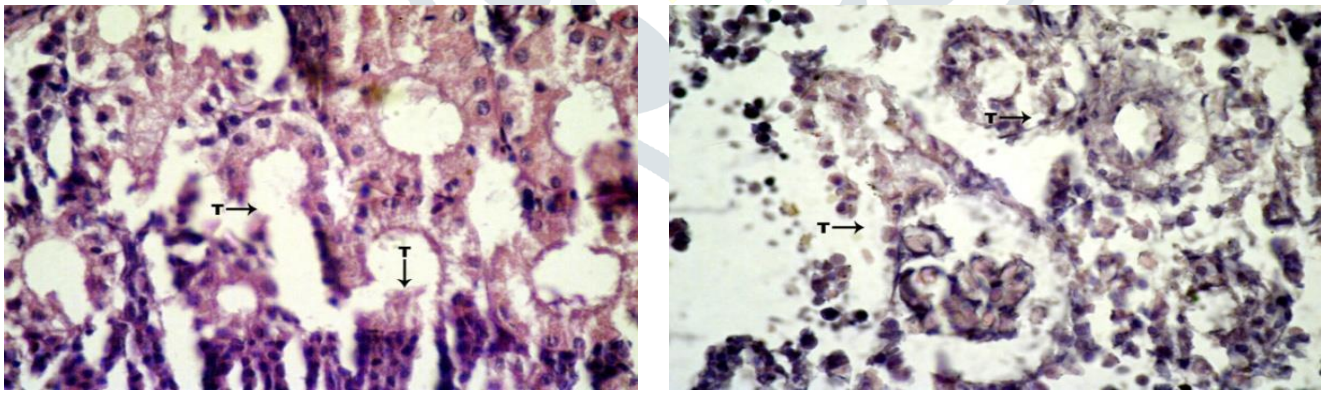
(a) (b)

Fig. 3: Photomicrographs of fish kidney (Mercury treated) showing evacuated glomerulus (a-0.10 mg/L and b-0.25 mg/L, 30 days) G (Glomerulus)



(a) (b)

Fig. 4: Photomicrographs of fish kidney (Mercury treated) showing evacuated glomerulus (a-0.40 mg/L and b-0.55 mg/L, 30 days) G (Glomerulus)



(a) (b)

Fig. 5: Photomicrographs of fish kidney (Mercury treated) showing damaged tubules (a-0.40 mg/L and b-0.50mg/L, 30 days) T (Tubule)

Similar observations have been reported earlier also upon exposure to various pollutants: Endosulfan (*Heteropnestus fossilis*- Bhatia and Sandhu, 2005), Cadmium (Wistar albino rats-Guberlay *et al.*, 2004 and *Mylio* macrocephalus (Teleostei)- Ooi and Law, 1989), Malathion (*Clarias batrachus* (Linn)- Bhatnagar *et al., 1993)* Mercury compound (*Clarias batrachus* (L.)- Kirubagaran and Joy, 1988), Elsa (*Channa punctatus*- Banerjee and Bhattacharya, 1994), DDT (Coho salmon – Buchlar *et al.*, 1969), 3, 3, 4 Triaminoazobenzene

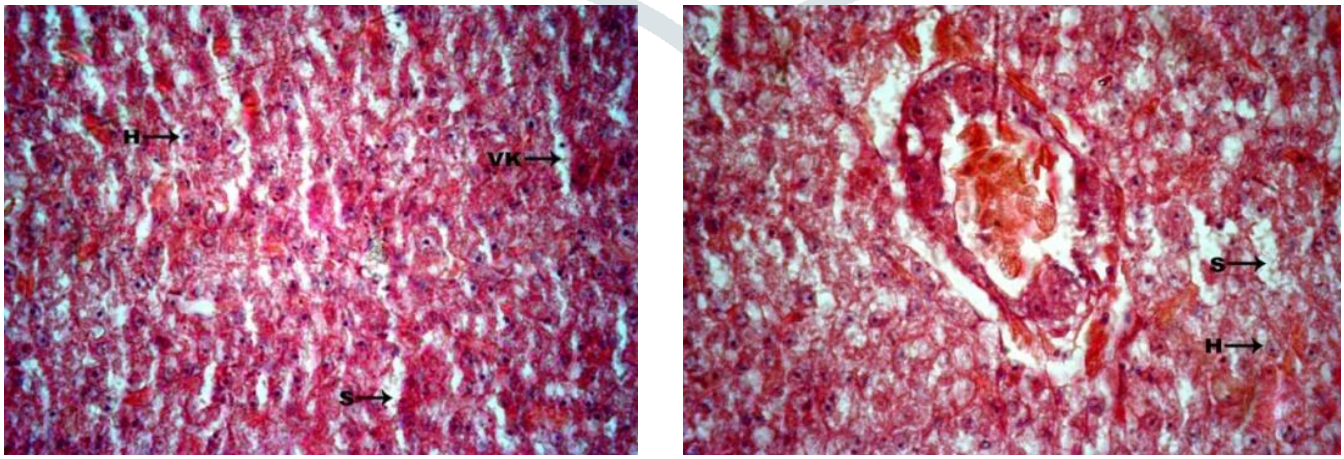
(*Channa punctatus*- Goel and Garg, 1977), Mercury (*Heteropneustus fossilis*- Bano and Hasan, 1990), Mercury, (*Poecilia reticulate*-Wester and Canton, 1992) Lindane (*Colisal fasciatus*- Verma *et al.,* 1975), Chlorphyrifos (*Hetropneustus fossilis* – Srivastva *et al., 1990*), Carbofuran (*Colisa lalia*- Sukumar and Karpaganapathy, 1986), Hydrothol-191(sunfish-Eller, 1969), Malathion (mice- Barlas and Kolankaya, 1996). Kidney tubule necrosis on toxicant exposure has been reported by Srivastava *et al.* (1990), Gill *et al.* (1988), Sukumar and Karpsgaganapathy (1986), Cassillas *et al*.(1983), Srivastava and Srivastava (1981), Kumar and Pant (1981).

Liver External morphology: Liver in *Channa punctatus* is bilobed, reddish brown and dense organ, which is located in the upper region of the body cavity. Right lobe is large and thick mass whereas left lobe is further subdivided into two lobes, anterior and posterior. The gall bladder is embedded in the right lobe. The vascular system of this organ consists of two afferent blood vessels (hepatic artery and hepatic portal vein) and a single efferent vessel (hepatic vein) located at the hilum region of kidney.

General Structure of Liver: The liver of *Channa punctatus* is made up of hepatic lobules which comprise of hepatic cells having located nucleus which contains a nucleolus and homogenous cytoplasm (Fig.6a). These parenchyma cells contain masses of glycogen, whose formation generally begins in the center of lobules while the secretions of bile occur at periphery. The hepatic cells are arranged in the form of hepatic cords which are two cells thick, but branching and anastmosing of cords often results in four or more cell layers (Fig. 6a). The hepatic cords are pierced with a network of sinusoids blood from interlobular branches of the portal vein. At intervals, in the wall of the sinuses are present conspicuous cells known as satellite cells or Von kupffer (VK) cells (Fig. 6a).

The traids are constituted by ramification of portal vein; hepatic portal artery and biliary duct are indistinct in *Channa punctatus*, as in other teleost. Hence, the term "portal regions" (Fig.6b) is more appropriate in place of portal triads as in mammals. On exposure period to 30 days the effects of lesions were seen (Figs. 7b; 8a and b and 9a and b). This was probably because of site-specific action of heavy metal thus impoverishing the cell of their various organelles. They also showed increases in the size of hepatic sinusoids. These effects were dose dependents. Vacuolization were also noticed, their size and number was concentration dependent (Figs. 10a and b; 11a).

Significant alterations were noticed in the portal region also. At lower concentrations (0.080 mg/L and 0.10mg/L, 30 days) degeneration of the wall of the portal region was observed (Fig. 11b). With increase in concentration (0.25 mg/L 0.40 mg/L and 0.55 mg/L, 30 days) both degeneration and evacuation of the portal region was noticed (Fig.12a & b, 13a and b).



H (Hepatocyte), S (Sinusoid), VK (Von kupffer cell) (a) (b)

Fig.6 : Photomicrographs of fish liver (control) showing (a) two cell thick cords of hepatocytes and sinusoid with Von kupffer cell (b) portal region of the liver along with hepatocytes.

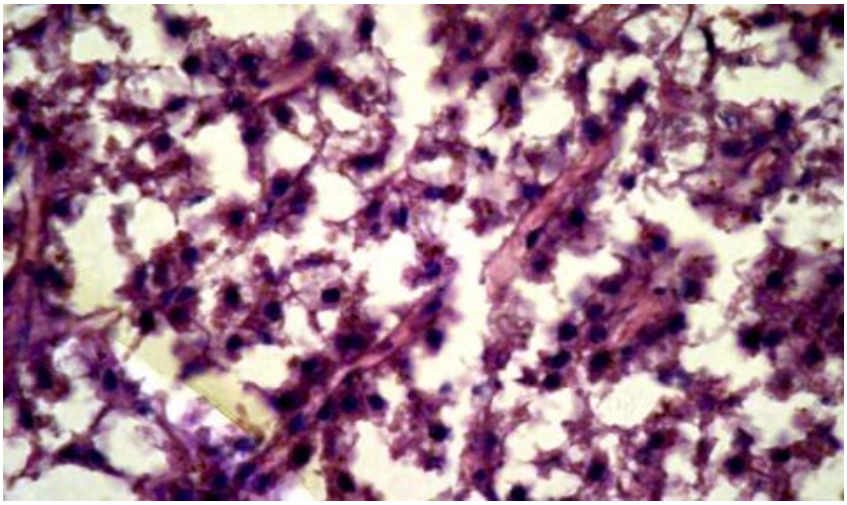


Fig.7 : Photomicrographs of fish liver (Mercury treated) showing degeneration of hepatic cell boundaries (a-0.080 mg/L, 30days)

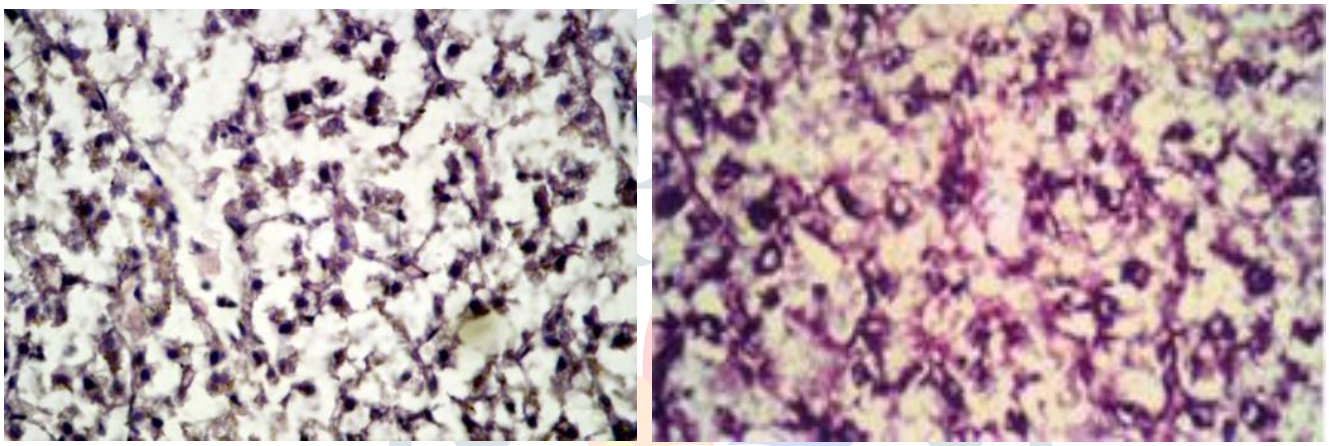


Fig. 8: Photomicrographs of fish liver (Mercury treated) showing degeneration of hepatic cell boundaries (a-0.10 mg/L and b-0.25 mg/L, 30days)

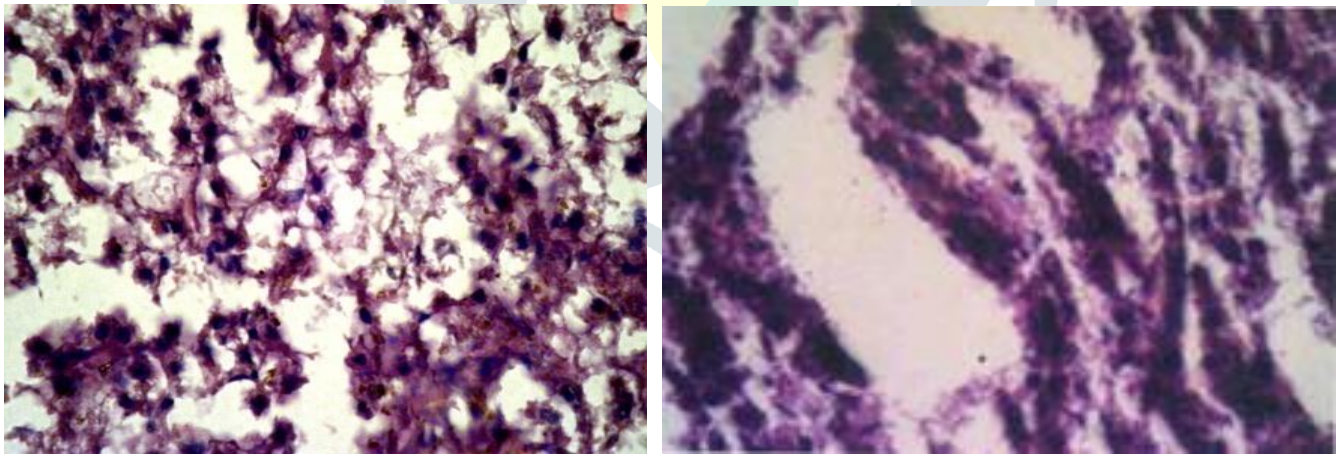


Fig. 9: Photomicrographs of fish liver (mercury treated) showing degeneration of hepatic cell boundaries and great increase in sinusoidal space (a-0.40 mg/L and b-0.55 mg/L, 30 days)

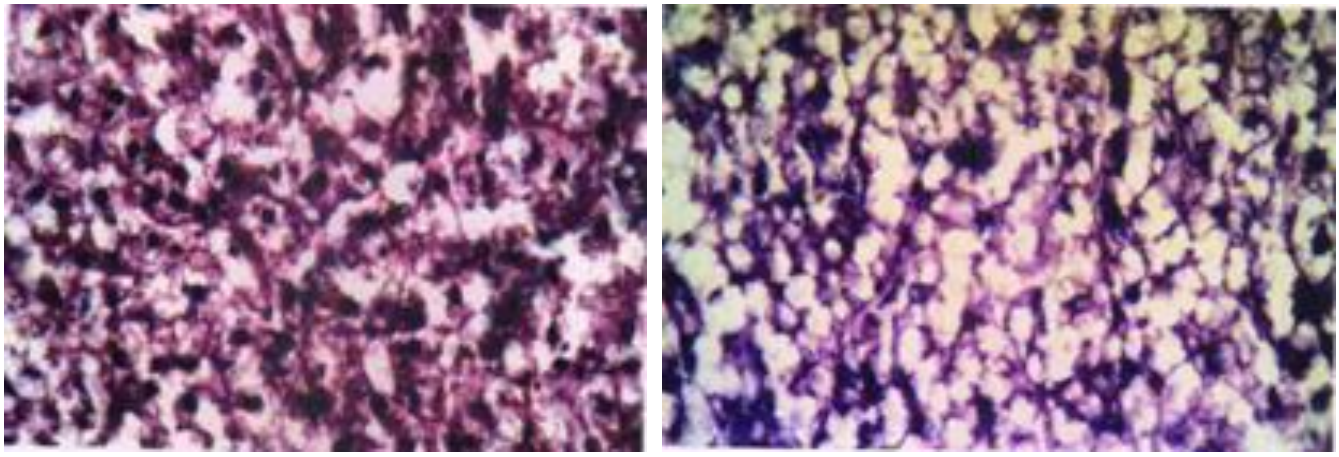


Fig. 10: Photomicrographs of fish liver (Mercury treated) showing much more increase in size of vacuoles (a-0.25 mg/L and b-0.40 mg/L, 30 days)

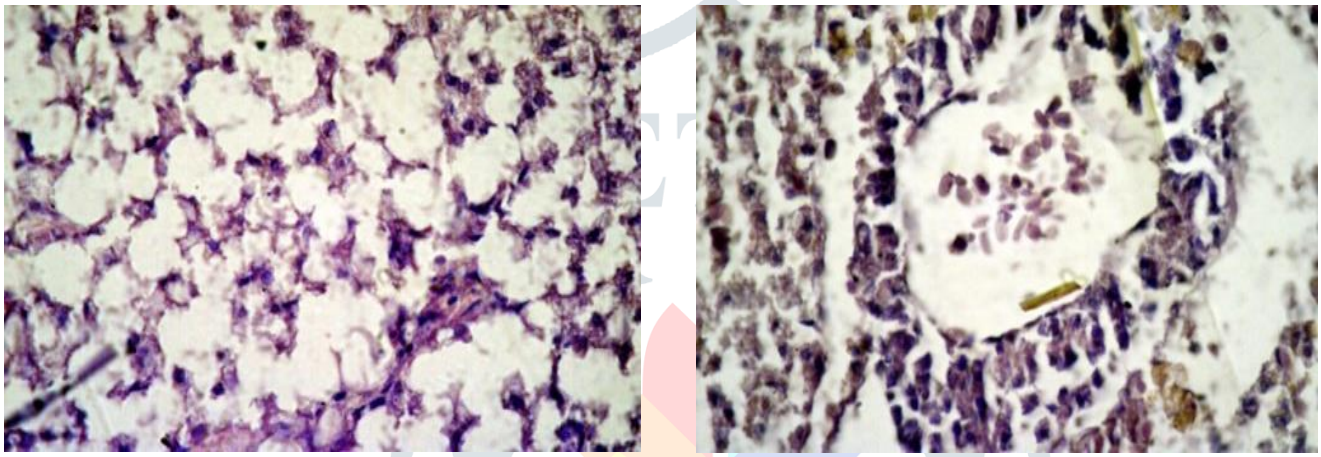


Fig. 11: Photomicrographs of fish liver (Mercury treated) showing (a) much more increase in size of vacuoles (0.55 mg/L and (b) degeneration of the wall of portal region (0.080 mg/L, 30 days)

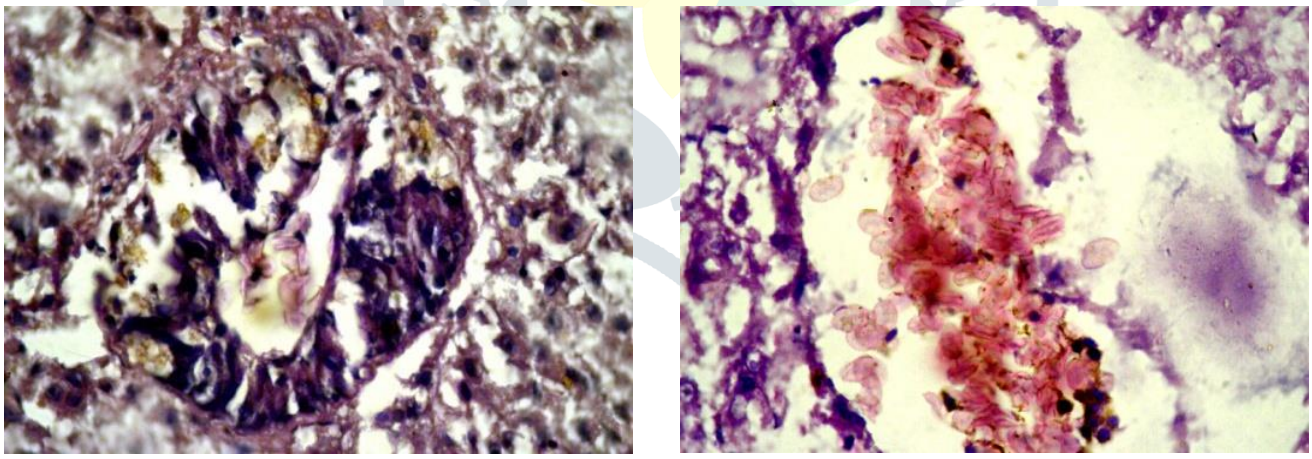


Fig. 12: Photomicrographs of fish liver (Mercury treated) showing degeneration and evacuation of the portal region (a-0.10mg/L and b-0.25 mg/L, 30 days)

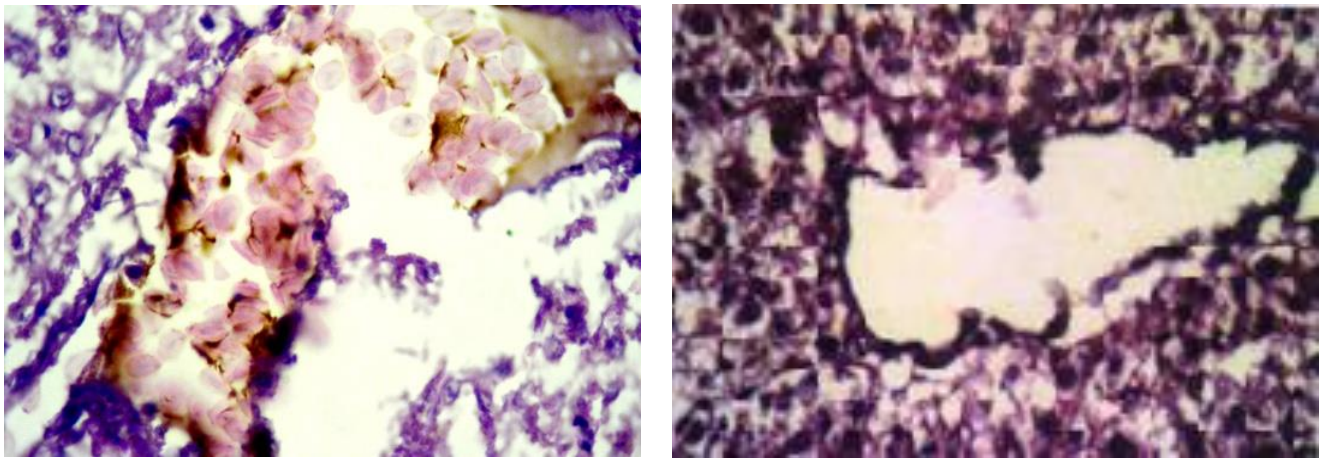


Fig. 13: Photomicrographs of fish liver (Mercury treated) showing totally degenerated and evacuated portal region (a-0.40mg/L and b-0.55 mg/L,30 days)

The present findings are in conformity with the earlier reports of Mathiessen and Roberts ; Gill *et al*; Asztalos *et al*] and Jonsson and Toledo. These workers reported vacuolation and hypertrophy in the hepatocytes; pycnosis; lytic necrosis; focal cell necrosis; lipid accumulation and the absence of nuclei in various regions of the liver parenchyma in various fish species, which are exposed to various toxicants. Through SEM, cytopathological alterations of hepatocytes such as irregular nuclei outlines and heterochromatin, fragmentation vesiculation of endoplasmic reticulum with electron dense bodies and lipid inclusions and increased serum metabolic enzyme activity were noticed in *Cyprinus carpio* under the stress of gallium The loss of various subcellular organelles and nuclei showed that Mercury has site-specific response, thus causing impoverishment of parenchymal cell of its vital constituents. The congestion in hepatic parenchyma and the irregular enlargement in the liver sinusoids also indicate the hazardous potential of this mighty chemical at cellular level.

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