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Histopathological alterations in the liver of *Channa punctatus* when exposed to Mercury

Neeraj Gupta[1*] and Anish Dua[2]

[1]Department of Zoology, DAV College, Amritsar, INDIA
[2]Department of Zoology, Guru Nanak Dev University, Amritsar, INDIA

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ABSTRACT

Live specimens of *Channa punctatus* were collected, after acclimatization they were chronically exposed to sublethal concentrations of mercuric chloride for 15 and 30 days. On exposure fish liver histology showed hepatic congestion and distinct cell boundaries were found to be destroyed. Liver also showed increases in the size of hepatic sinusoids. These effects were dose and duration dependents. With the increase of exposure period to 30 days the effects of lesions were also increased.

1) INTRODUCTION

Pollutants are the substances which pollute the environment by adding into it. Certain of them are such that once they are released they persist and harm the environment. Agricultural runoff, industrial wastes, household wastes and commercial properties etc. are the enormous sources of these pollutants [1]. Water pollutants are one such category; once they are released they harm the aquatic biota. 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 [2]. 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 [3].

Gandhi and Kumar [4] and Sambyal *et al*, [5] made a study on the residents of village Mahal noticed DNA damage and higher frequency of micronucleated cells caused by the chronic exposure to water polluted by heavy metals released by industrial effluents of that region.

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 [6]. 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 histopathology of liver in fresh water fish *Channa punctatus.*

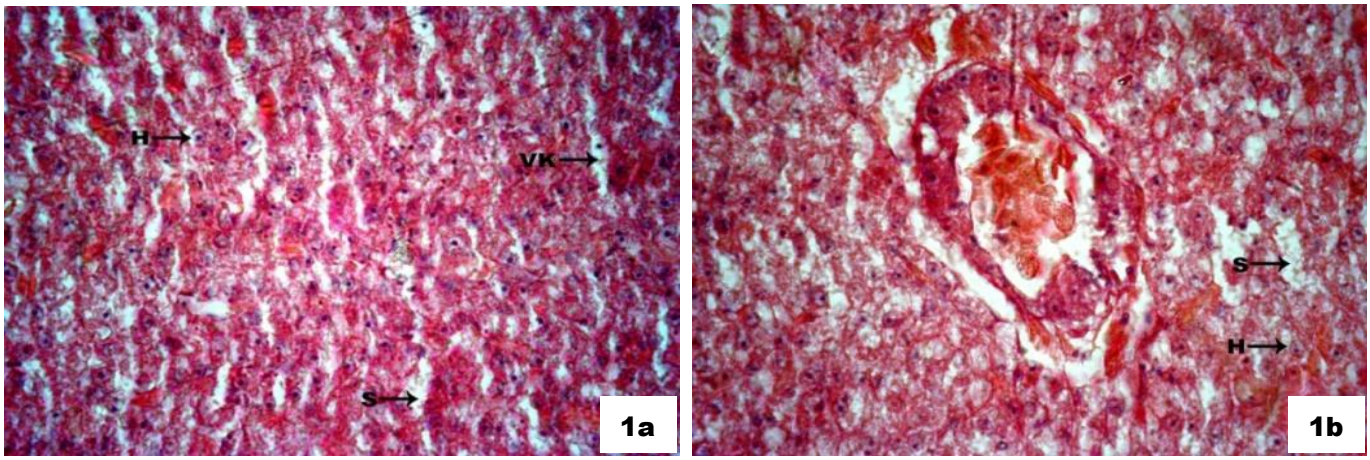
2) MATERIALS AND METHODS

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 [7]. 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 15 days and 30 days.

3) RESULT AND DISCUSSION

* Corresponding Author: **Dr. Neeraj Gupta**
Email address: neerajgupta9474@gmail.com



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

Fig. 1: 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.

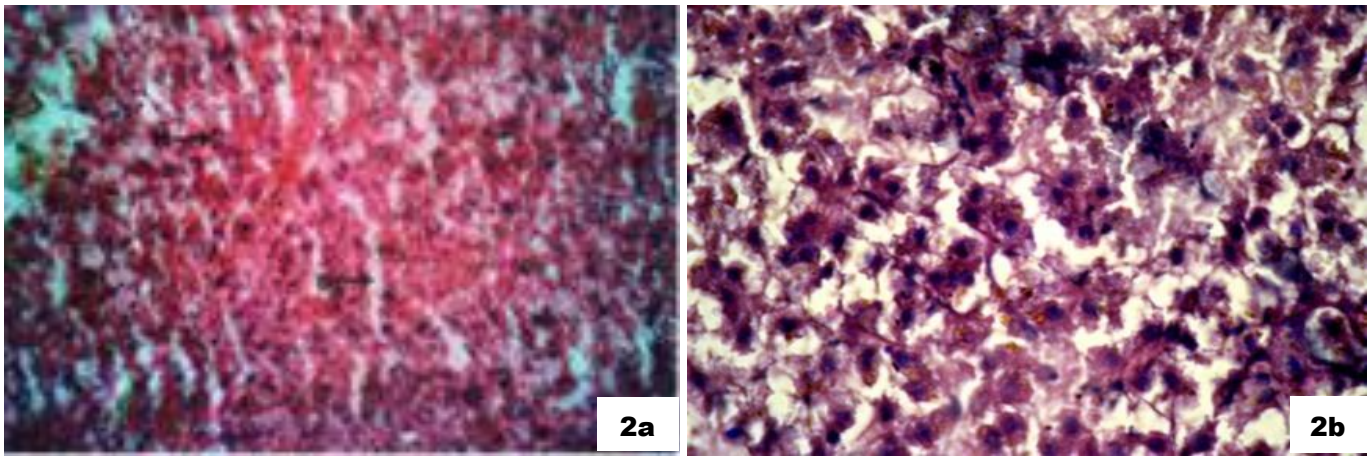


Fig. 2: Photomicrographs of fish liver (Mercury treated) showing (a) hepatic congestion (0.080mg/L, 15 days) (b) degeneration of hepatic cell boundaries (0.10 mg/L, 15 days).

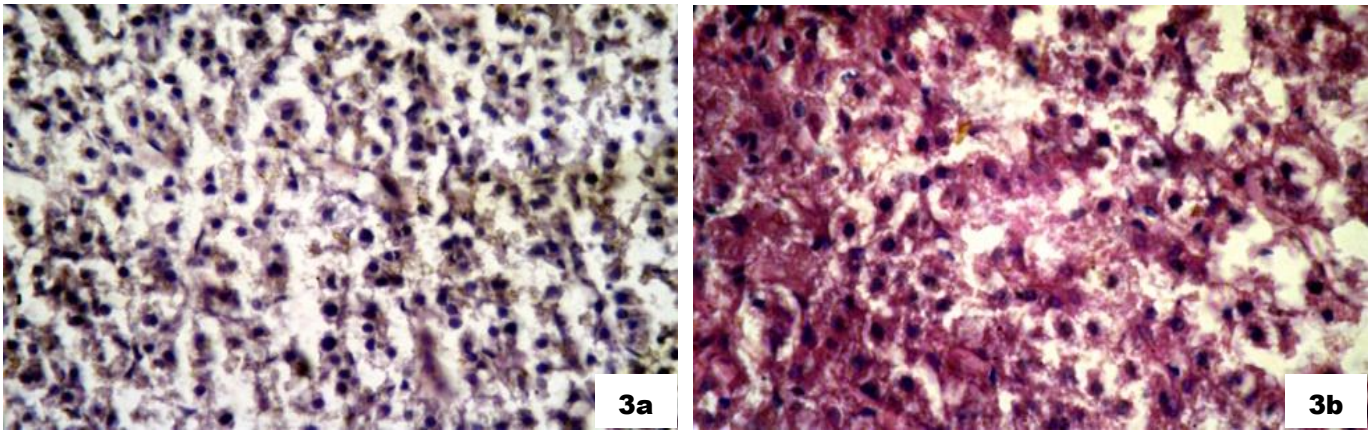


Fig. 3: Photomicrographs of fish liver (Mercury treated) showing degeneration of hepatic cell boundaries (a-0.25 mg/L and b- 0.40mg/L, 15 days)

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. The liver size is expressed as hepatosomatic index (HIS = Liver weight X 100/body weight) which is very low in air breathing fishes. HIS of female *Channa punctatus is higher i.e.* 0.914± 0.041 as compared to male which is 0.863±0.05 [8]

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.1a). 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. 1a). 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. 1a). These cells lie in the contact with liver cells and are highly phagocytic in nature. They also have a tendency to take in red blood cells which appear to undergo disintegration

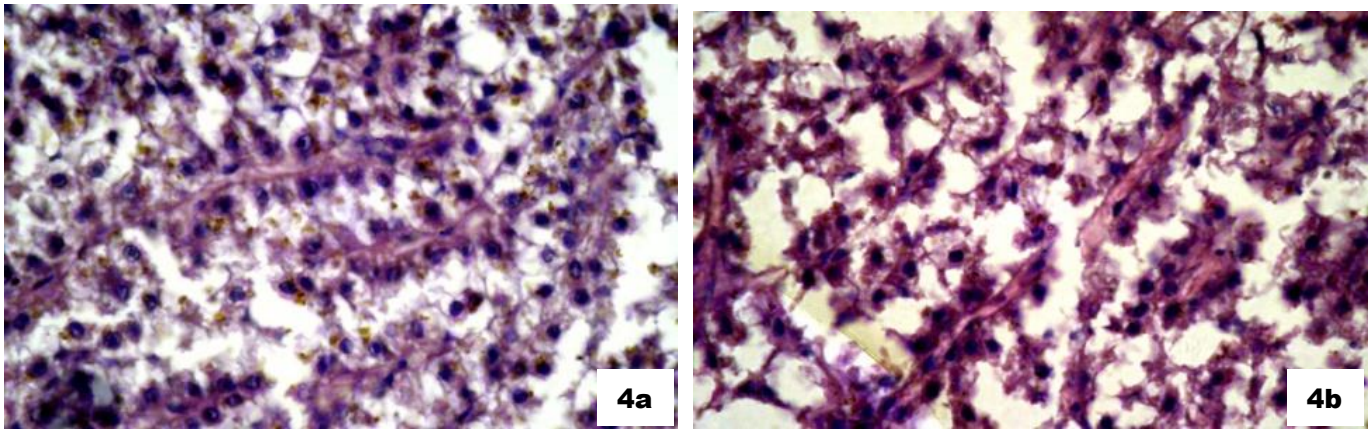


Fig. 4: Photomicrographs of fish liver (Mercury treated) showing degeneration of hepatic cell boundaries (a-0.55 mg/L, 15 days. b-0.080 mg/L, 30days)

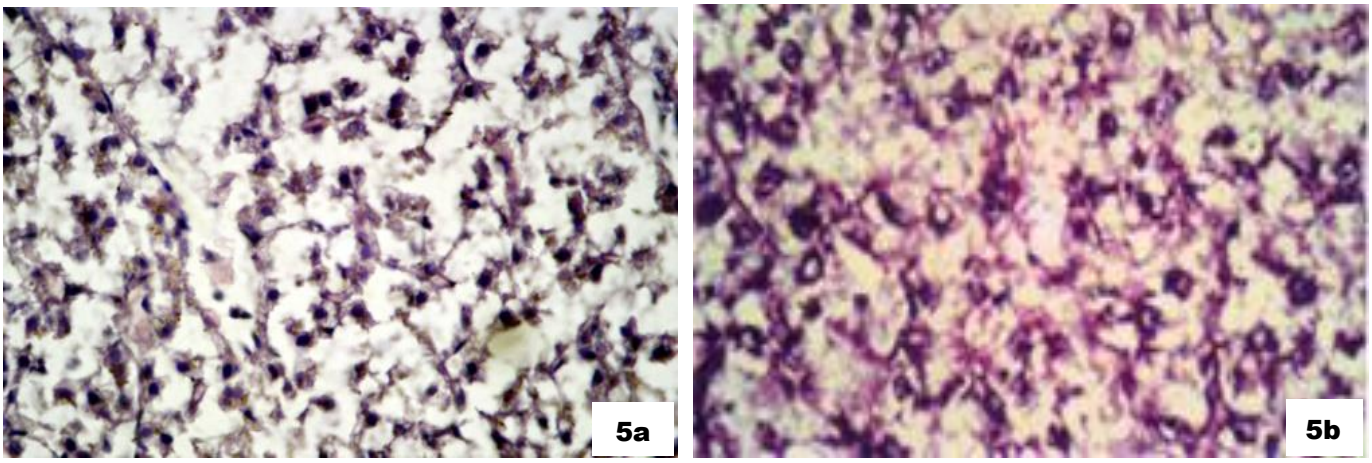


Fig. 5: Photomicrographs of fish liver (Mercury treated) showing degeneration of hepatic cell boundaries (a-0.10 mg/L and b-0.25 mg/L, 30days)

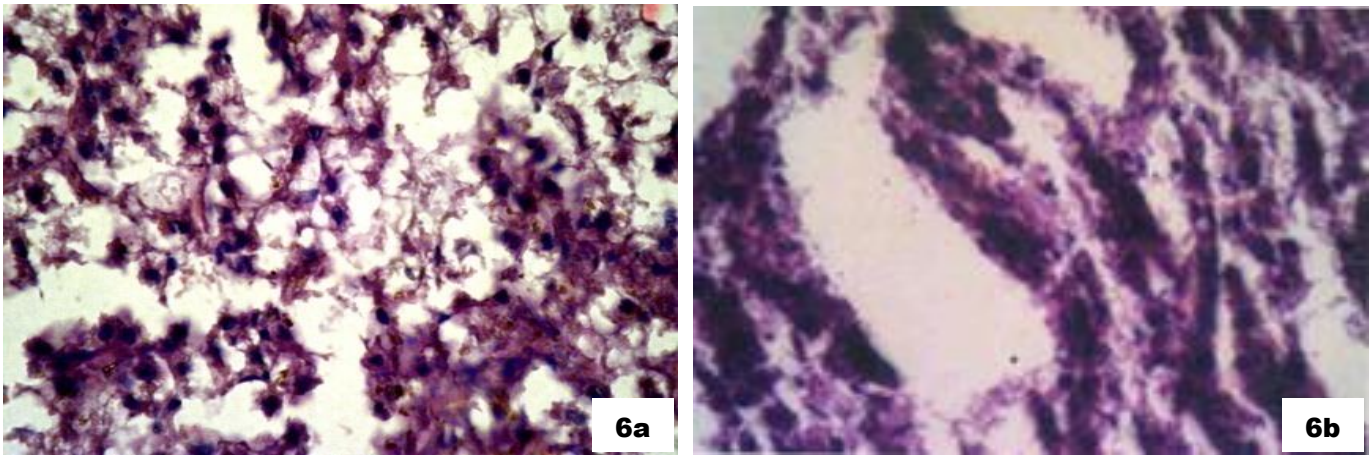


Fig. 6: Photomicrographs of fish liver (mercury treated) showing degeneration of hepatic cell boundaries and great increase in sinusoidal space (a-0.40 mg/L and b-0.55 mg/L, 30 days)

within them. Bile pigments are formed as a result of this process [9].The traids are constituted by ramification of portal vein; hepatic portal artery and biliary duct are indistinct in *Channa punctatus*, as in other teleost [10]. However, some triads have been reported in Caranx spp and Lutajens bohar. Hence, the term "portal regions" (Fig.1b) is more appropriate in place of portal triads as in mammals, when referring to fish's liver. Hepatic veins distantly located at the centre at the hepatic lobules (also called central lobular vein) were found randomly throughout.

On exposure to various sublethal concentrations of Mercury for 15 and 30 days, the hepatic tissue show marked histopathological alterations. Fish showed hepatic congestion (0.080 mg/L, 15 days) (Fig.2a) and distinct cell boundaries

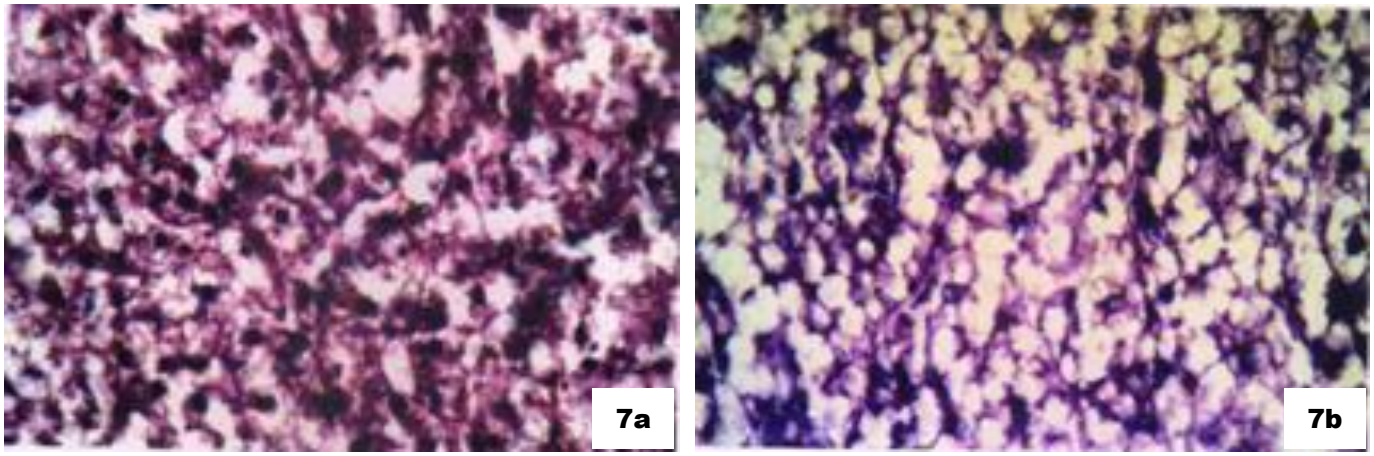


Fig. 7: Photomicrographs of fish liver (Mercury treated) showing much more increase in size of vacuoles (a-0.25 mg/L and b-0.40 mg/L, 30 days)

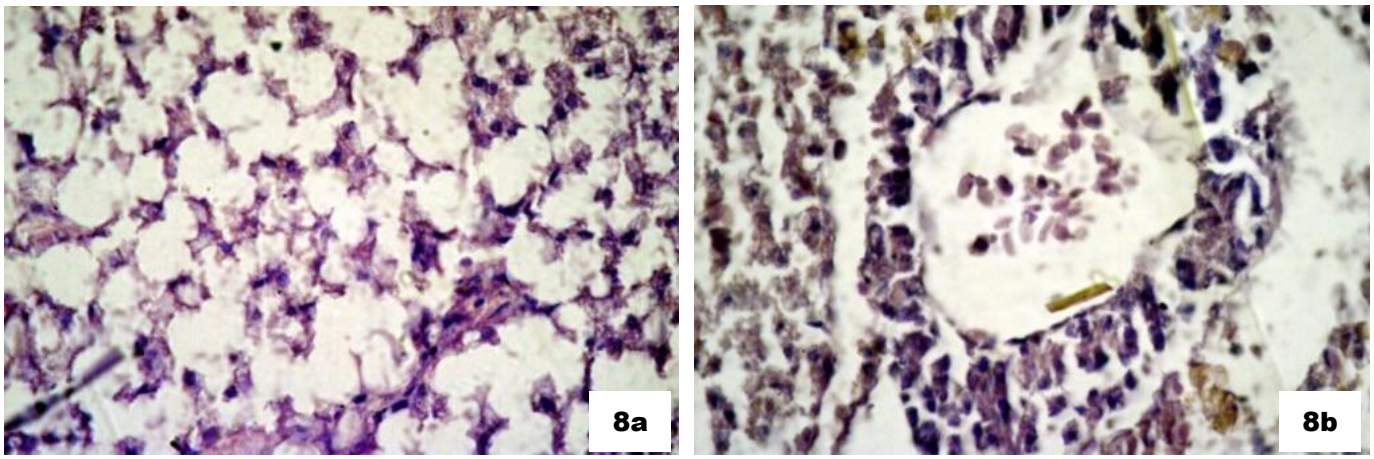


Fig. 8: Photomicrographs of fish liver (Mercury treated) showing (a) much more increase in size of vacuoles (0.55 mg/L and (b) degeneration of the wall of portal region (0.080 mg/L, 30 days)

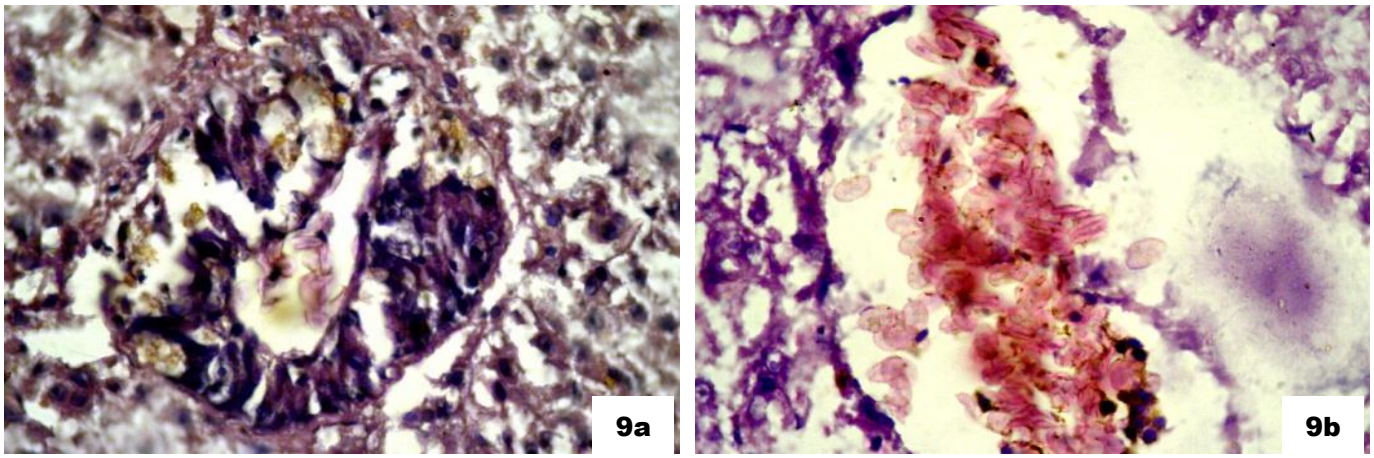


Fig. 9: Photomicrographs of fish liver (Mercury treated) showing degeneration and evacuation of the portal region (a-0.10mg/L and b-0.25 mg/L, 30 days)

were found to be destroyed, when exposed to higher concentrations (0.10 mg/L to 0.55mg/L, 15 days) (Figs. 2b; 3a and b 4a). They also showed increases in the size of hepatic sinusoids. These effects were dose and duration dependents. With the increase of exposure period to 30 days the effects of lesions were also increased (Figs. 4b; 5a and b and 6a and b). This was probably because of site-specific action of heavy metal thus impoverishing the cell of their various organelles.

By increasing the exposure period for 30 days vacuolization were also noticed, their size and number was concentration dependent (Figs. 7a and b; 8a and b and 9a).

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. 8b and 9a). 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. 9b and 10a and b).

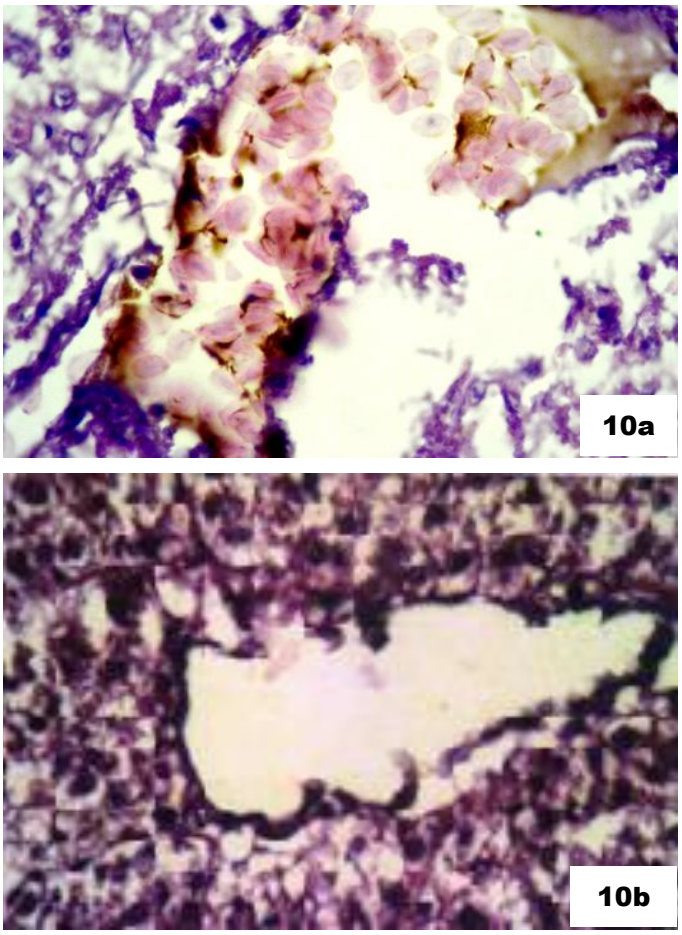


Fig. 10: Photomicrographs of fish liver (Mercury treated) showing totally degenerated and evacuated portal region (a-0.40mg/L and b-0.55 mg/L,30 days)

The present findings are in conformity with the earlier reports of Mathiessen and Roberts [11]; Gill *et al*,[12]; Asztalos *et al.*[13] and Jonsson and Toledo[14]. 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, disruption of mitochondria, proliferation of lysosomes with electron dense bodies and lipid inclusions and increased serum metabolic enzyme activity were noticed in *Cyprinus carpio* under the stress of gallium [15] Tzirogiannis *et al.* [16] noticed liver tissue necrosis, apoptosis and inflammatory infilitration when treated with Cadmium toxicity in male wistar rats. They also noticed recovery of all these alteration when rats were retreated with normal saline. 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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