WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.045
Volume 3, Issue 10, 1196-1204. Research Article ISSN 2277– 7105

EFFECT OF HEAVY METAL INDUCED HISTOPATHOLOGICAL ALTERATIONS IN LIVER OF CLARIAS BATRACHUS (LINN)

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Article Received on
03 Oct 2014,

Revised on 29 Oct 2014,
Accepted on 24 Nov 2014

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ABSTRACT

Discharge of heavy metals into aquatic environment from various sources even below permissible levels, creates health hazards in aquatic organisms. The persistence and ubiquitous nature of these pollutant compounds coupled with their tendency to accumulate in organisms ultimately produce toxic reaction in aquatic biota especially, fish. This study aims to investigate histopathological impact of lethal concentration (1.0625 ppm, 1.4202ppm) and sublethal concentration (0.1062ppm, 0.0531ppm and 0.1420ppm, 0.0710ppm) concentrations of zinc sulphate and copper sulphate in liver of fresh water teleost,Clarias batrachus(Ham). The histopathology studies revealed vacuolation in cytoplasm, degeneration of nuclei, vacuolation in stroma, cloudy swellings, pycnotic nuclei, necrosis, rupture of blood sinusoids, disarray of hepatic cords, loss of shape of hepatocytes. Severity of damage was found to be dose dependent and time of exposure.

KEYWORDS: Histopathology, Heavy metals, necrosis, cloudy swelling, disarray, Clarias batrachus.

INTRODUCTION

The contamination of fresh water with a wide range of pollutants has become a matter of concern over the last few decades (Vutukuru, 2005; Dirilgen, 2001; Voegborlo et.al,1998). The natural aquatic system may extensively be contaminated with heavy metals released from domestic, industrial and other man made activities (Velez and Montoro, 1998; Conacher et al 1993). Heavy metal contamination may have devasting effect on the ecological balance of the

recipient environment and diversity of aquatic organisms (Farombi et al, 2007; Vosyliene and Jankiite, 2006; Ashraj, 2005).The chief source of contaminants are industrial waste discharge, mining, agriculture, household waste disposal and fuel combustion (Woodling et al 2001;Patra et al 2005 and Swarup et al 2006; Saxsena and Garg, 2011). It appears that problems of heavy metal accumulation in aquatic organisms including fish needs continuous monitoring and surveillance owing to biomagnifying potential of toxic metals in human food chain (Das and Kaviraj, 2000; Laxi,2005; Jayakumar Paul, 2006; Kumar et al 2007,08) Accumulated heavy metals may lead to morphological alterations in the tissues of fish (Monteiro et al 2005). Histopathology deals with the study of pathological changes induced in the microscopic structure of the body tissue. Any peculiar type of alteration of cells may indicate the presence of the disease or the effect of toxic substance. Thus study of histopathology is of prime importance in the diagnosis, etiology and prevention of disease. In fishes, it is observed that the external organs are affected due to toxic chemicals, causing loss of equilibrium, increase in opercular movements, to and fro irregular vertical movements, finally leading to death. This may be attributed to the significant damage to the internal organs. Histopathological study thus gives us useful data concerning tissue change prior to external manifestation.

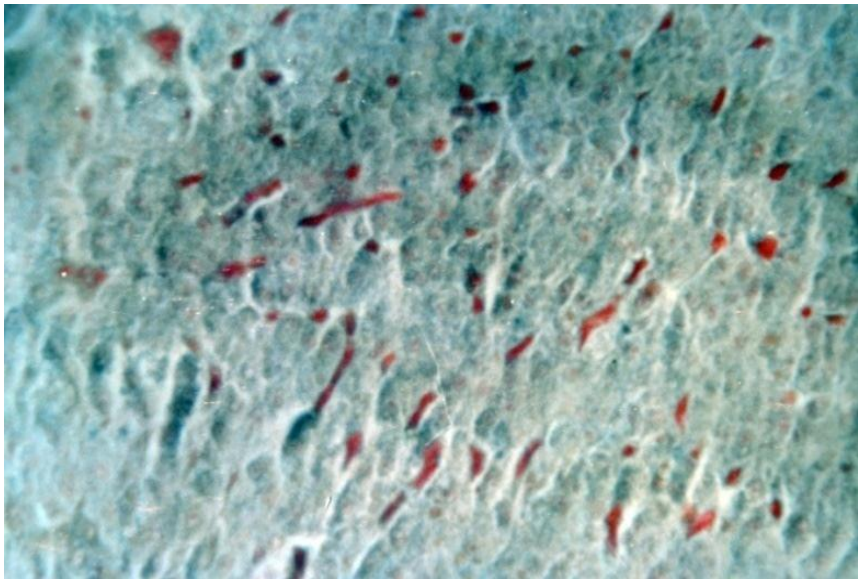
MATERIALS AND METHODS

Adult and live fish *Clarias batrachus* were collected from the farm Patra and Bhadbhada Bhopal brought to the laboratory, cleaned by using 0.1% $KMno_4$ to avoid dermal infection. Only healthy fishes (Length: 12-15cm, Weight: 50-60g) were taken for experiment. Fishes were acclimatized in glass aquaria for 15 days and were fed with fish food (earthworms) and water in the aquaria was replaced by freshwater at every 24h. Fish showing normal activities were selected for each test. In first set fish were exposed to 96 hours LC50 concentration of mercury chloride (1.062 ppm) and copper chloride (1.4202 ppm) . Above values of lethal concentrations are obtained in earlier work. At the end of acute exposure the survived fish were decapitated and immediately liver tissue was removed and fixed in aqueous Bouin's fluid for 24 hours. This tissue was dehydrated in different alcohol grades and blocks were prepared in paraffin wax (580 to 600c). The sections of 5G to 6G were cut and stained with Mallory's triple stain (Mallory 1944) and mounted in DPX. The same procedure was repeated for liver tissue of control fish as well as fish exposed to sublethal concentrations of mercury chloride and copper chloride. Sublethal concentrations were $1/10^{th}$ and 1/20th of 96 hrs LC50

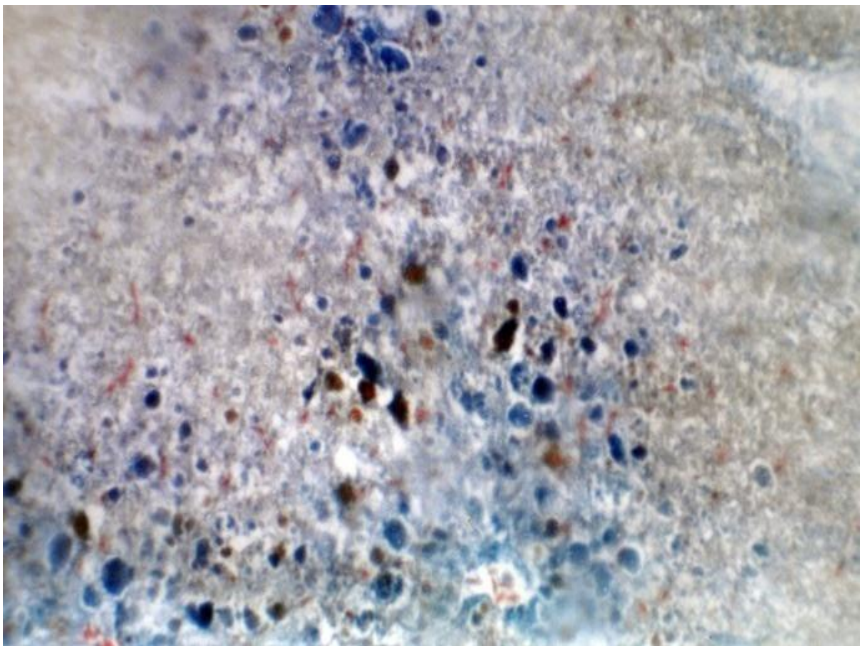
concentrations of zinc sulphate (0.1062 and 0.531 ppm) and copper sulphate (0.1420 and 0.0710 ppm).

RESULTS

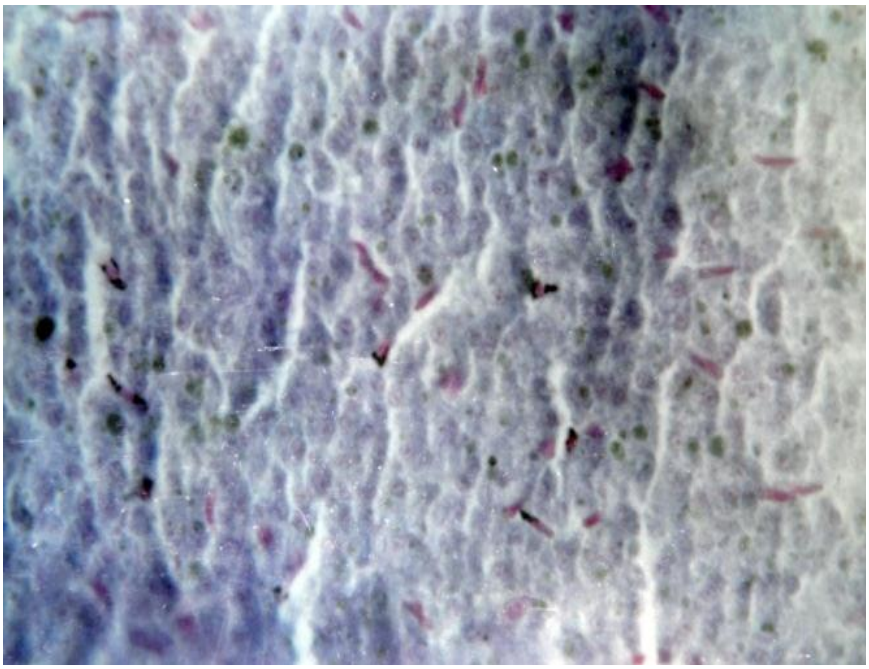
Metabolism of food items, their storage and detoxification are the important functions of liver. Toxic substances reach liver through blood. Hence liver is susceptible to number of toxic substances and metabolic distributions. The histopathological changes observed in present investigation after exposure to lethal and sublethal concentrations of mercury chloride and copper chloride in the liver of test fish *Clarias batrachus* have been depicted in photo plate 1 (B to E). The liver of the fish exposed to heavy metal compounds exhibited marked histopathological alterations. Liver of test fish *Clarias batrachus* exposed to lethal concentrations (96 hrs LC50 concentrations) of zinc sulphate and copper sulphate showed vacuolation in the cytoplasm, degeneration of nuclei, vacuolation in stroma. The alterations in liver of fish exposed to sublethal concentration of zinc sulphate were, cloudy swellings of the cells with large vacuoles, degeneration of nuclei, vacuolation in stroma, pycnotic nuclei, shifting of nuclei on one side of the cell, prominent necrosis. The changes observed due to exposure to sublethal concentrations of copper sulphate include rupture of blood sinusoids, disorganised (disarray) hepatic cords, loss of shape of hepatocytes. Severity of damage was more in copper sulphate exposed fish than zinc sulphate exposed fish. It was also found to be dose dependent and time of exposure.



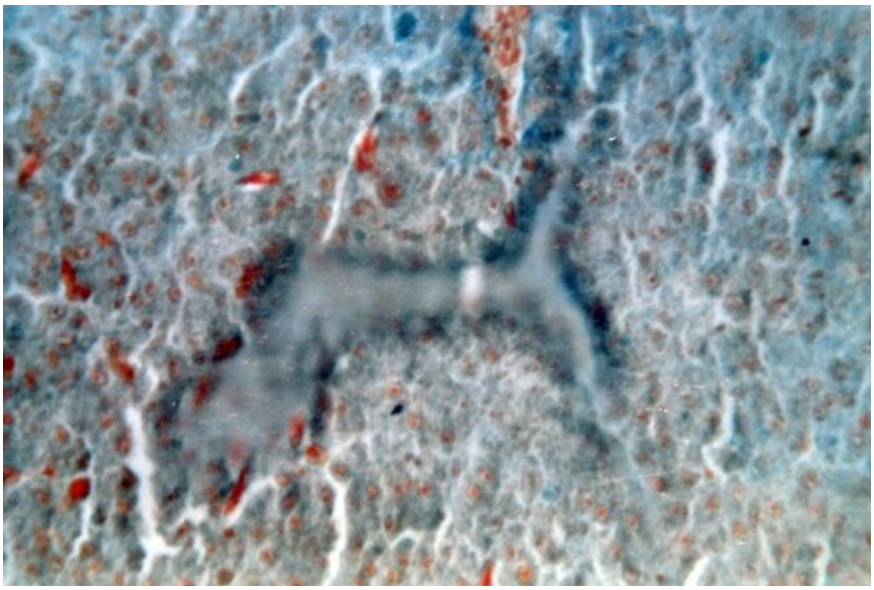
A. Micro photograph showing T.S liver of *C. batrachus* Control T×400



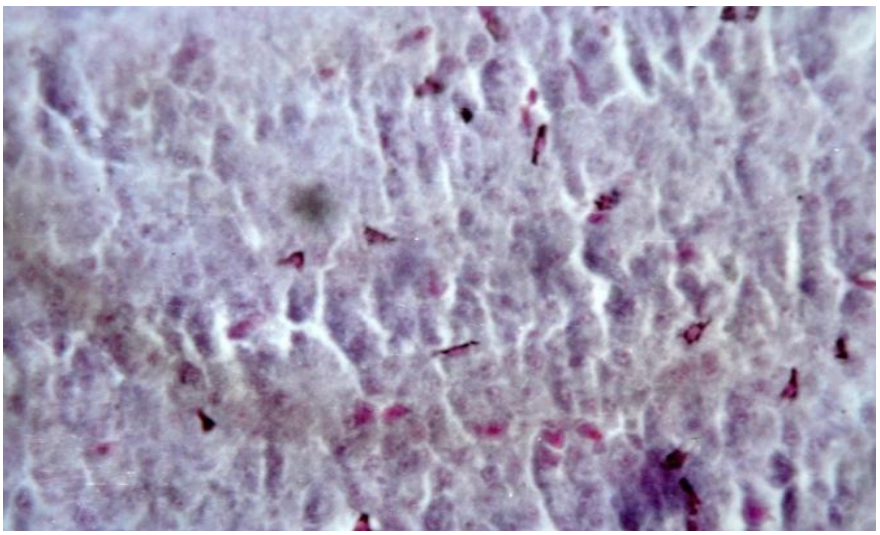
B. Micro photograph showing T.S liver of C. *batrachus* 96 hrs exposure to zinc sulphate (1.06 ppm) MT×40



C. Micro photograph showing T.S liver of C. *batrachus* 96 hrs exposure to copper sulphate (1.47 ppm) MT×400



D. Micro photograph showing T.S liver of C. *batrachus* 45 days exposure to zinc sulphate (0.106 ppm) MT×400



E. Micro photograph showing T.S liver of C. *batrachus* 45 days exposure to copper sulphate (0.147ppm)MT×400.

DISCUSSION

The toxicity effect of heavy metals and pesticides on liver have been studied by many workers. Sastry and Gupta (1978) reported liver cord disarray, shrinkage in the liver cells, degenerated nuclei and focal necrosis in *Clarias batrachus* due to lead intoxication. Benedetti *et.al* (1981) reported cytoplasmic vacuolation in hepatocytes of liver of Ictaburus nebulous due to copper pollution. Kumar and pant (1981) studied vacuolation within and outside the hepatocytes, severe necrotic changes in liver, breakdown of cellular boundary, vacuolation in liver of Puntius conchonius induced by copper and zinc intoxication. Singh (1983) reported

vacuolation and necrosis in liver of Colisa fasciatus exposed to copper sulphate. Dalela et.al (1984) reported necrosis, hypertrophy and atrophy in the liver tissues, loss of polygonal shape of liver cells, splitting of the cells and formation of spaces in the tissues after exposure of Cyprinus carpio to lethal and sublethal concentration of copper and cadmium. Bakre (1985) reported cellular damage, nuclear hypertrophy of hepatocytes, vacuolation and necrosis leading to lysis, increase in blood sinuses, bile canliculi and bile pigments become prominent in Gambusia affinis on exposure to mercury chloride. Ram and Sathyanesen(1987) reported hyperplasia, nuclear pycnosis, fatty necrosis, degeneration of hypatocytes leading to tumor and Sycytium formation, blood vessel congestion, oedema, marked reduction in hypatosomatic index in Channa punctatus exposed to mercurial fungicide. Ramlingam (1988) reported necrosis, fatty degeneration, red blood cell occlusion in portal vessels, engorged blood vessel congestion, vacuolar degeneration of hepatocytes, in the liver of Sartherodon mossambicus. Khangarot (1992) reported cellular necrosis, clumping of chromatin and its aggregation at the centre, loss of nuclear membrane of hepatocytes after exposure to copper in Channa punctatus. Roncero et al (1992) reported intense hemolysis, massive necrosis liver parenchyma after acute exposure to copper sulphate in Tinca tinca L. Similar histopathological alterations were observed by Figueiredo et al (2007) in liver of Oreochromis niloticus exposed to copper, Grosell M et al (1996) in tissues of Anguilla anguilla on exposed to copper, Roganovic et.al (1998) in liver of Rutilus rubiliohridanus from heavy metal contaminated lake, Roganovic et.al (2003) in hepatic capillaries in Barbus meridionalis (Petenyiheck), Varanka et al (2001) in liver of Cyprinus carpio L on exposure to copper sulphate.

CONCLUSION

Heavy metals like mercury and copper enter the aquatic ecosystem through a wide spectrum of natural source such as volcanic activities, erosion and anthropogenic ones including industrial wastes as well as a leakage and get further biomagnified in the food chain. Histopathological alterations in fresh water test fish under the influence of heavy metals can be used as a sensitive model to monitor the aquatic pollution. The current result indicates that the heavy metal contamination definitely affect the liver showing vacuolation in cytoplasm, in stroma, degeneration of nuclei cloudy swelling of the cells, pycnotic nuclei shifting of nuclei on one side of the cell. Prominent necrosis, rupture of blood sinusoids, disarray of hepatic cords, loss of shape of hepatocytes. Hence a scientific method of detoxication is essential to improve the health of these economic fish. The present research work served as

an experimental tools and bioindicators for the first line evaluation of environmental pollution.

ACKNOWLEDGEMENT

Authors are beholden to Principal Dr. A.K. Gangely S.L.L. Jain P.G. College Vidisha, Bhopal M.P. India for providing laboratory facilities for this research work. This paper forms the part of Ph.D. thesis submitted by author to Barkatullah University, Bhopal M.P. (India).

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