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*RESEARCH ARTICLE*

# TOXIC EFFECTS OF CU($SO_4$) ON GILL AND LIVER TISSUES OF FRESH WATER CATFISH *CLARIAS BATRACHUS* (LINN.).

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## *Abstract*

Heavy metals are a major problem because they are toxic and tend to accumulate in living organisms. This study was carried out with the aims of studying on histopathology of Cu($SO_4$) toxicity on gill and liver tissues of catfish *Clarias batrachus* within the period of 96 h. Totally, 140 fishes with mean weight 60 ± 10 g were stocked in 12 aquariums with capacity of 200 L water and divided in to 3 trails including control, 0.3 ppm and 0.5 ppm of Cu with 3 replicates. Tissue samples were fixed by bouin's solution and sectioned in 7 µm based on histological regular method and stained with Hematoxylin and Eosin (H & E) method for microscopic study within the period of 96 h. Results showed some damaged such as hyperplasia, telangiectasis and edema, necrosis of second filaments, jerky movement, aneurism, hyperaemia and fusion of second filaments in gills; and cell atrophy, necrosis, fatty degeneration, hyperaemia and bile stagnation at different treatments in comparison with control. Gill and liver tissue damages were severed with the increase of Cu concentration and days. Therefore, Cu had acute toxicity effects on gill and liver tissues in Catfish at 0.3 and 0.5 ppm concentrations.



## Introduction:-

Heavy metals are serious danger for the earth ecosystem. Accumulation of heavy metals in water, air and soil is a major environmental problem. A little amount of copper is produced from destroying of soils and rocks (Kasraiee, 2009). Naturally, heavy metals formed lower than 1% of body weight and their concentration fluctuations lead to environmental impermanent and disaster in animals (Clark, 1986). Industrial development and environment chemical pollution threatens aquatic animal life. Environmental pollutions with heavy metals were increased in the world and it may be bioaccumulated in fish tissues (Mansour and Sidky, 2002). Therefore, heavy metals are the most important pollutant of aquatic ecosystem that is the cause of major problems for human (Karan *et al*., 2002). Some metals such as copper and ferric are needed in little amount for natural development, but some of them such as Cu, Cd, Pb and Hg are toxicant at lower concentrations. Therefore, discharge of industrial, mineral, agricultural, house sewage and fuel (Swarup *et al*., 2006; Patra *et al*., 2005; Woodling *et al*., 2001), algaecides and fungicides used in aquaculture (Onwumere and Oladimeji, 1990) are considered as aquatic ecosystem pollutants so that

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industrial wastewaters are the main source of pollution (Kaviraj and Das, 1995) that include various kinds of toxic pollutants such as suspended solid, minerals, poisons and pesticides (Kumar and Singh, 2010). Therefore, heavy metal pollutions have harmful effects on environmental equivalent and animal diversity (Vinodhini and Narayanan, 2008). Copper is one of these pollutants and also added to ponds as a micronutrient for increasing in production of planktons and fish (Adhikari and Ayyappan, 2004). Catfish is a warm water fish that feed on planktons. Copper may be transferred by plankton to fish and human finally. Higher amount of zinc leads to pathologic disasters in tissues and causes fish death. Some studies were done in Iran that showed the effects of Cu on different tissues of *Cyprinus carpio* (Mohammad Khanlo Ashaieri, 2003; Rostami *et al*., 2000; Rostami and Soltani, 2009) in rainbow trout (*Oncorhynchus mykiss*) (Farangi and hajimoradloo, 2007) and in fingerlings of *Acipenser persicus* (Moshtaghi *et al*., 2009; Fathollahi *et al*., 2010). Therefore, the aim of this study was investigating histopathological effects of Cu acute toxicity on gill and liver tissues in *C. batrachus.*

## Materials and Methods:-

Adult and live fish *C. batrachus* were collected from the fish farm Patra and Bhadbhada Bhopal M.P.) brought to the laboratory, cleaned by using 0.1% $KMno_4$ to avoid dermal infection. 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.

140 fishes with mean weight 60 ±10 g in 3 treatments (0.3 and 0.5 ppm) with 3 replicates were stocked in aquarium with capacity of 200 L water. Firstly, the fishes were adapted to clinical conditions and then introduced to detected concentrations. Desired concentrations of Cu were measured by the Germ/volume method and using C2V2 = C1V1 formula. Firstly, the total required Cu was measured and then dissolved in a specific volume of the whole water for providing stock solution. Then, specific volumes of the stock were poured into aquariums. Samples of gill and liver tissues were collected at 24, 48, 72 and 96 h, fixed by Bouin's solution and dehydrated based on standard method, clarified, embedded, sectioned with 7 µm diameters by using microtome set (model Letiz 1512, Germany), stained based on Hematoxylin and Eosin (H & E) method (Hallajian, 2010) and were studied by light microscope.

## Results:-

Clinical studies results showed some apparent signs such as fast opening and closing of operculum and mouth, fidgety and air swallowing.

**(A) Microscopic study of gill:-**

Some damages including hyperplasia, telangiectasis, and edema, necrosis of second filaments, jerky movement, aneurism, hyperemia and fusion of second filaments in gills at 0.3 and 0.5 ppm concentrations than control and severe with increase of concentration and days

**(B) Microscopic study of liver:-**

Microscopic studies of liver samples showed some damages such as cell atrophy, cell necrosis, fatty degeneration, hyperemia, bile stagnation and melanomacrophage at 0.3 and 0.5 ppm than control (Figures 4, 5 and 6). Liver damages were lower at 0.3 ppm than 0.5 ppm of Cu. Therefore, with the increase of zinc sulphate concentration and days up to 72 h liver damages were severe but at 72 & 96h.

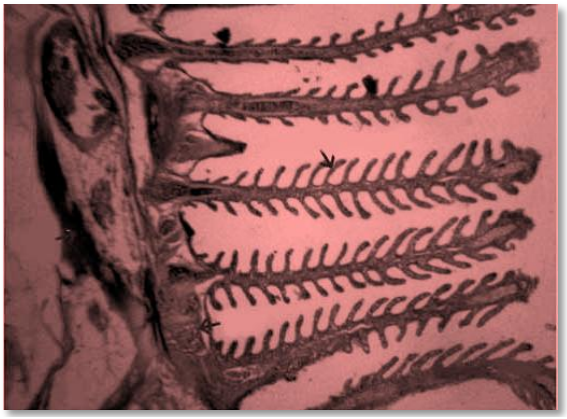

**Figure 1:-** part of control gill showing structural organization. Note the gill lamella, taste bud and gill arch. H/E x100

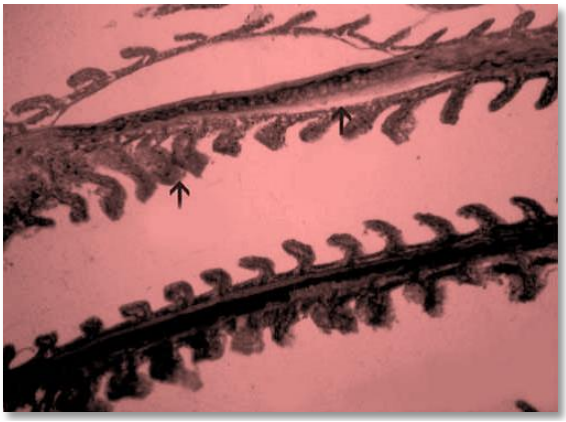

**Figure 2:-** Hyperplasia (H), fusion of gill filaments (F), at 0.3 ppm of Cu after 12 h. (H & E, 245X).

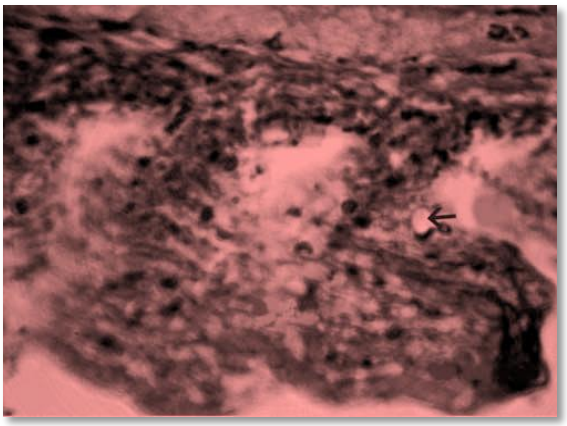

**Figure 3:-** Hyperplasia, haemosiderin (He), fusion of filaments (F) and necrosis of cells (N) at 0.5 ppm at 96 hrs. H & E, 245X.

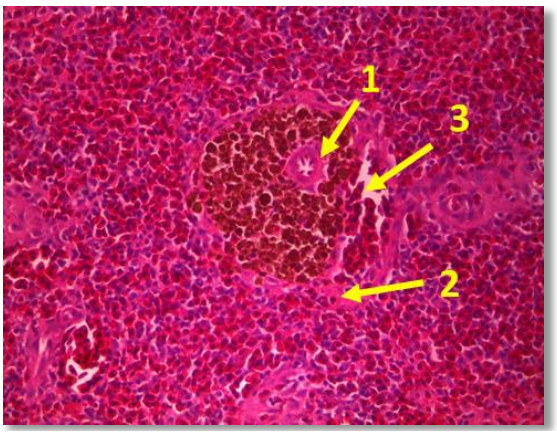


**Figure 4:-** Liver cells at control (H&E, 750X).

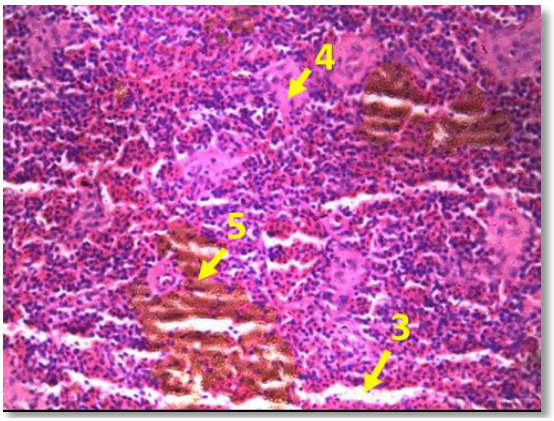


**Figure 5:-** Hyperemia (H), bile stagnation (Bs) (H&E, 750X).

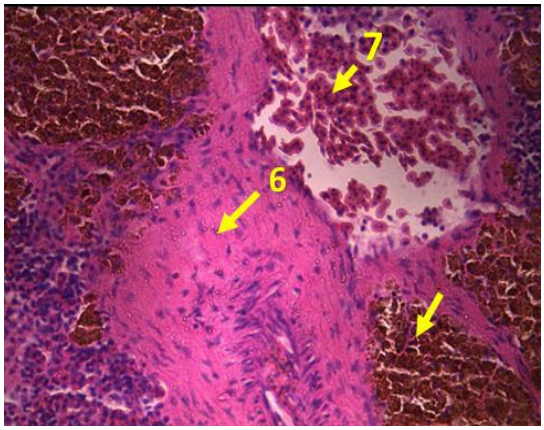


**Figure 6:-** Necrosis of cells (N), fatty degeneration at concentration (F) (H&E, 750X).

## Discussion:-

Heavy metals have an important physiological effects and causes of many diseases if decreased or increased. Copper is a mineral ingredient and its toxicity has bad effects on the body of fishes (Donmez *et al*., 1993). Gill structure in Catfish includes primary and second lamella. Secondary lamellas of this fish are long and free from the end of distal. A normal secondary lamella complex was from two thin layers of epithelial cells, blood vessels and row of cells (Athikesavan *et al*., 2006). Copper has effects on chloride cells, gill morphology and physiology such as pH reduction in blood vessel, decrease in oxygen absorption and heart beat and delays egg hatch time and increase of larvae survival. Cu is resistant to deposition and lingering in environment for long time. Acute damages caused by Cu on gill include lamella fusion, hyperplasia, hyperemia, cell necrosis and telangiectasis after 12 h. These disasters in fish are kind of responses to environment conditions, so that fusion of secondary filaments is due to mucosa glycoprotein on cells (Chreck and Moyle, 1990) which lead to increase exposure of lamella with air or oxygen for collapse and adhesiveness, hyperemia and hyperplasia that indicated an immune response to chemical matters such as heavy metals (Rostami *et al*., 2000). Hypertrophy and hyperplasia caused to disaster in gill epithelium cells because of reduction in water flow between gill filaments that decreased respiratory function (Marioara *et al*.2009). Some similar effects have been reported by Marioara *et al*. (2009) and Athikesavan *et al.* (2006) about Cadmium effects on Silver Carp; Naji *et al*. (2007) about Cu effects on Common Carp; Gorouiee *et al*. (2008), Colins and Brown (1998), and Jeney (1992) about aluminum sulfate effects on *Rutilus frisikuttum*, *Onchorhynchus mykiss* and *Cyprinus carpio*, respectively. Fernandez *et al*. (2008) indicated that long term exposure to heavy metals cause respiration, blood circulation and osmoregulation disasters in fishes. Alvarado *et al*. (2006) observed that high increase in chloride cells of gills led to thickness in epithelial cells, increase in migration of chloride cells into secondary lamella edge, hypertrophy and fusion of secondary lamella of gills. Liver play a main role in detoxication. Histological studies of liver showed cell atrophy, cell necrosis, hyperaemia and bile stagnation after 12 h exposure to Copper sulphate. Rostami *et al.* (2000), and Rostami and Soltani (2009) reported cell necrosis, increase of

*Hemosydrine* in Common Carp exposed to Cu. Kamaraju and Ramasamy (2011) indicated that increase in $NiCl_2$ concentration lead to decrease in glycogen level. Yilmaz *et al.*, (2011) showed that with increase of $CdSO_4$ concentration in *Leuciscus cephalus*, some disasters in liver such as fatty degeneration, cell necrosis and kopfer cells were severed according to present study results.

## Conclusion:-

Based on obtained results at present study, zinc sulphate induced some histopathological disasters and leads to fish death. Toxicity rate of Cu in fishes was severe with the increase of concentrations and days.

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