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STUDIES ON THE HISTOPATHOLOGICAL EFFECT OF ZINC SULPHATE ON GOLD FISH *CARASSIUS AURATUS*

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ABSTRACT: Purpose: Heavy metal in the aquatic environments has been as a potential threat to the aquatic organisms including fishes. Metals are known to inhibit the several biochemical and physiological mechanism vital for fish metabolism. In recent years metal concentrations were found to be raised in aquatic environment, due to discharge from industrial waste, agriculture and urban sewage. As a result, aquatic organisms are exposed to elevated levels of heavy metals in the coastal environment. Hence, the aim of the present study was to evaluate the effect of zinc sulphate on gold fish *Carassius auratus* under laboratory condition. Method: The experiment was carried out with three groups. Group I was kept as control which is free from zinc sulphate and the remaining group II and III were supplied with sub-lethal concentration of zinc sulphate 10 mg/l and 15 mg/l respectively. Fishes were exposed to these concentrations for 15 days. After the durations, the brain, liver, gills and ovary were dissected out and fixed in 10% formalin for histopathological study. Hematoxylin and Eosin were used to stain nucleus and cytoplasm of the cells. Stained slides were observed under research microscope and photographs were taken. Results: The histopathological studies have revealed that severe damage have been noticed in gills followed by liver, brain and ovary tissue, because gills come in immediate contact with environment. The destructive changes have been directly related to both concentration of doses and the period of exposure. Conclusion: The heavy metal toxicity has to be controlled or else it would affect the aquatic ecosystem and in turn other natural resources and causes toxicity to plants, animals and humans.

Keywords: Gold fish *Carassius auratus*, Zinc sulphate, Histopathology, Toxicity, Brain, Liver.

I. INTRODUCTION

Heavy metals pollution is a major ecological concern due to its high persistence in the environment. The agricultural and industrial activities are the main source of heavy metal pollution, which adversely affect the aquatic ecosystem (Rashed 2001; Yilmaz 2003; Khare and Singh 2002). Although aquatic ecosystems are equipped with a variety of physico-chemical and biological mechanisms to eliminate or reduce adverse effects of toxic substances, toxicants may evoke changes in development, growth, reproduction and behavior or may cause death of freshwater organisms (Eisler, 1993). The sources of heavy metal pollutants are industrial wastewater from mining, metal processing, tanneries, pharmaceuticals, pesticides, organic chemicals, rubber and plastics, lumber and wood products. The heavy metals are transported by runoff water and contaminate water sources downstream from the industrial site. To avoid health hazards, it is essential to remove these toxic heavy metals from waste water before its disposal (Srivastava *et al.*, 2006).

Mercury, lead are known to be for their toxicity, whereas Mn, Cu, Zn, Se, Cr, and Co are essential elements (Bazzi *et al.*, 2008). Hg is noted for its neuro toxicity and at high doses; it can cause serious damage to the central nervous system, brain and kidney. Low doses may have some development effects and infants exposed via the mother's diet (Holmes *et al.*, 2009). Heavy metal toxicants are accumulated in the fish through general body surface which affects severely their life support system. Once this toxic substance enters into body, they damage and weaken the mechanism concerned leading to psychological and biochemical disorders (Arasta *et al.*,1999).

Behavior is considered a promising tool in ecotoxicology. The use of behavioral changes in fishes are diagnostic end point, for screening and differentiating chemicals according to their mode of action. Chemicals are categorized to corresponding three general mode of Action Response Syndrome; Hyperactivity, Hypoactivity and Physical Deformity (Drummond *et al.*, 1990). Zinc is one of the most common heavy metal pollutants. The sources of zinc and other heavy metals in natural waters may be from geological rock weathering or from human activities such as industrial and domestic wastes water discharges and animals where it forms constituent functions. It maintaining cytoplasmic integrity (Weatherly *et al.*, 1980). However, at high concentrations, zinc adverse effect in fish accruing structural damage, which affects the growth, development and survival of fish (Tuurala and Soivio,

1982). Zinc accumulations in the gills of fish and this indicates a depressive effect on tissue respiration. The study of histopathology is of primary importance in the diagnosis, etiology and prevention of disease. Data on tropical fish and effects on different fish tissues are still scarce (Mela *et al.,* 2007). Metal toxicology including accumulation and damage in the different target organs. The liver is a vital organ concerned with basic metabolism and is the major organ of accumulation, biotransformation and excretion of contaminants in fish (Figueiredo *et al.,* 2006). Hence, in the present study an attempt has been made to investigate on the histopathological effect of lead acetate in various organs such as gills, liver, brain and ovary of gold fish *Carassius auratus*.

II. MATERIALS AND METHODS

2.1 Selection of fishes

old fish *Carassius auratus* (a fresh water ornamental fish) was selected for the present study. Male and female gold fish are purchased from Local fresh water aquarium.

2.2 Acclimatation of Gold fishes Under Laboratory Condition

Before starting the experiment, gold fishes were maintained in large plastic tub containing dechlorinated water. Totally 25 gold fishes were introduced. They are supplied with artificial fish feed pellets. The fishes were monitored for a week. Once in two days the water was changed. After 7 days out of 25 gold fishes, healthy 15 fishes were separated from the stock and taken for experimental studies. Before introducing them in the experimental setup initially the fishes were weighted, length and breadth were measured. 15 fishes were used for further experimental purpose.

2.3 Preparation of Zinc sulphate

For the present study Zinc (Zn2+) was chosen as heavy metal toxicant. The zinc was given in the form of Zinc sulphate dissolved in distilled water. Two sublethal concentrations i.e: 10mg/l and 15mg/l of zinc sulphate were selected for the present study.

2.4 Water analysis

Physiochemical parameters of water were analyzed as per methods given in APHA *et al.*, 2005.

2.5 Experimental set up

The three round shaped plastic tubs (capacity 20 litres) were selected for the present experiment. Before starting the experiment, the tubs were cleaned well. Water was drained out and cleaned thoroughly with fresh water, then allowed to dry for one or two hours and each tub was filled with 10 litres of tap water. The experiment was carried out with three groups (I, II, III). In each group five gold fish were introduced. Each group I, II III was provided with electric motors, for aeration. In general, they are very active swimmers and need a lot of swimming and hiding space in the tub, hence large tubs were kept. All fishes were fed with artificial fish feed pellet. Group I was kept as control which is free from heavy metal toxicant zinc sulphate and the remaining group II and III were supplied with sublethal concentration of 10 mg/l and 15 mg/l of zinc sulphate respectively (Fig. 1a-c). Fishes were exposed to these concentrations of zinc for 15 days. Fishes were fed with artificial feed as pellet.

(a) Group I **(b) Group II** **(c) Group III**

Fig.1. Experimental groups of gold fishes *Carassius auratus.*
(a) Group I – Control; (b) Group II - 10 mg/l conc. of zinc sulphate; (c) Group III – 15 mg/l conc. of zinc sulphate

2.6 Histological methods

After 15 days the fishes were sacrificed the brain, liver, gill, and ovary from group I (control), group II and9 III were dissected out, and fixed in 10% formalin. After fixation of organs, they were washed twice with distilled water to remove the excess fixative. Dehydration is done from lower grade of alcohol to higher grade of alcohol such as 30%, 50%, 90% and 100%. The time in each grade of alcohol was adjusted according to the thickness of the organs. The organs were embedded in paraffin wax and sectioning was done using the microtome. 4-7µ thickness sections were made and slides were prepared. Then the slides were stained using Hematoxylin and eosin to stain nucleus and cytoplasm of the cells. Stained slides were observed under low (10x) (45x) and high magnification (400x) in the research microscope and photographs were taken.

III. RESULTS AND DISCUSSIONS

Fishes are extensively used to assess the health of aquatic ecosystem and their physiological changes serve as biomarkers to monitor the environmental pollution. The physicochemical parameters of water such as pH, Dissolved oxygen, Alkalinity, and Total hardness were analyzed in the present study and given in Table 1. The quality of water is important. Since it is main factor determining the suitability of water for culture of fishes.

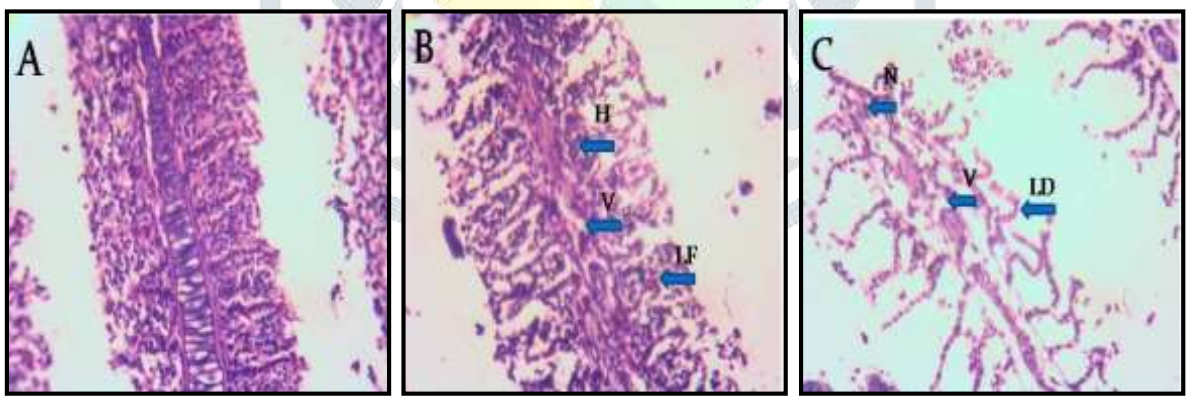
Table 1: Physiochemical parameters of water used for the experiment

S. No	Parameters	Values
1.	pH	7.96
2.	Total Hardness mg/l	220 mg/l
3.	Alkalinity mg/l	472 mg/l
4.	Dissolved oxygen	6.5 ml/l
5.	Temperature	29°C

The present study revealed that Gold fish *Carassius auratus* treated with metals manifested histopathological changes in gills, liver, brain and ovary.

3.1 Histopathological changes in the gills of gold fish *Carassius auratus*

In the control group I of gold fish the gill tissues showed normal structure. The gill tissues comprised of primary lamella (PL) and secondary lamellae (SL) and wide channel (WC). The secondary lamellae with single layer of epithelial cells were noticed. The photomicrograph of gills at 10mg/l concentration of zinc sulphate treated group II gold fish showed intensive lamellar fusion and vacuolation. Hyperplasia condition occurred and resulted in enlargement of gill tissues. The photomicrograph of gill at 15mg/l concentration of zinc sulphate treated group III of Gold fish showed intensive vacuolation. Severe lamellar damage has occurred. The structure of gills was totally damaged. Necrosis at center of primary lamella of the gill was observed (fig. 2a-c). Jana and Bandopadhaya (1987) also noticed the lamella epithelial lining react to dissolved zinc creating tissue osmoregulatory imbalance. According to Sappal *et al*., (2009) the gills play an important role for the absorption of zinc from the water and hence the physiological and morphological changes often appear in fishes.

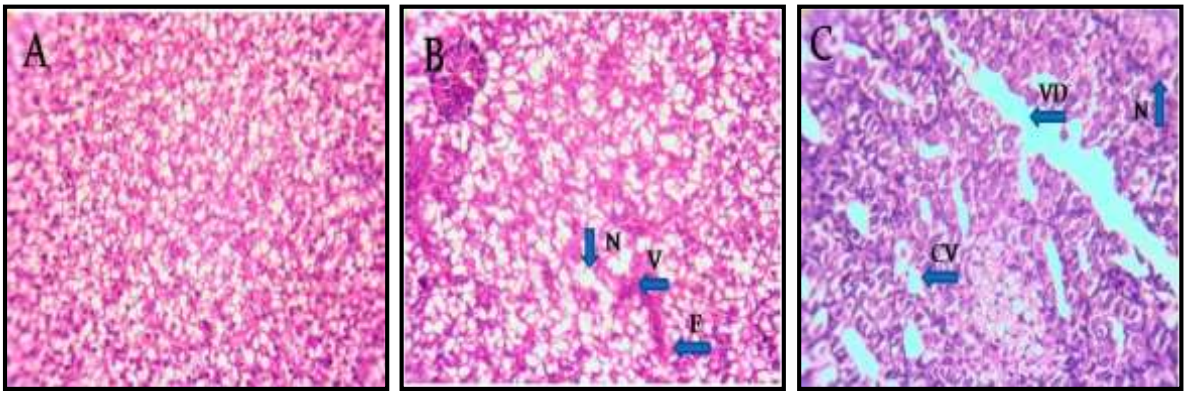


(a) Group I **(b) Group II** **(c) Group III**

Fig. 2: Photomicrograph of Gills shows Gold fish *Carassius auratus* (400x) (a) Group I Control; (b) Group II – 10 mg/l conc. of Zinc sulphate; (c) Group III – 15 mg/l conc. of Zinc sulphate. Hyperplasia (H); Vacuolation (V); Lamellar fusion (LF); Necrosis (N); Lamellar damage (LD).

3.2 Histopathological changes in the Liver of Gold fish *Carassius auratus*

The liver of Control Group I Gold fish was composed of hepatocytes arranged in typical tubular architecture. Normal cytoplasm with a large spherical nucleus was observed. The photomicrograph of liver at 10mg/l concentration of zinc sulphate treated Group II Gold fish showed fibrosis formation. Intensive hepatocellular vacuolation and necrosis were observed. Hepatopancrease with hyperlemic condition was noticed. The photomicrograph of liver at 15mg/l concentration of zinc sulphate treated Group III Gold fish exhibited severe necrosis and vascular dilation. Large cytoplasmic vacuoles were also noticed. At 15mg/l concentration, intensive hepatocellular vacuolation and hepatopancrease in the hyperlemic condition were noticed. This finding coincides with Dutta *et al*., (1993), Ortiz *et al*., (2002) who have suggested that morphological and histopathological alteration related to pesticides compounds caused severe damage to the liver cells.

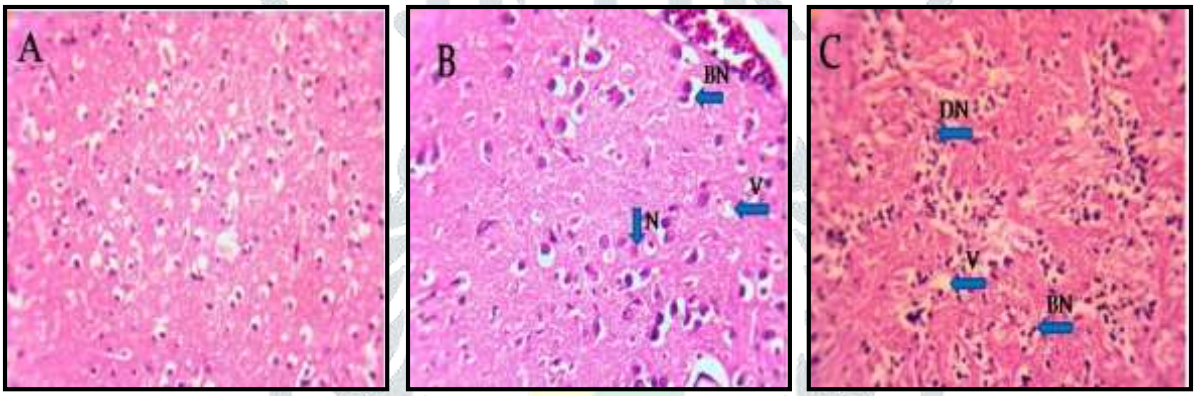


(a) Group I **(b) Group II** **(c) Group III**

Fig. 3: Photomicrograph shows Liver of Gold fish *Carassius auratus* (400x). (a) Group I – Control; (b) Group II – 10 mg/l conc. of Zinc sulphate; (c) Group III – 15 mg/l conc. of Zinc sulphate. Necrosis (N); Vacuolation (V); Fibrosis (F); Vascular dilation (VD); Cytoplasmic vacuolation (CV).

3.3 Histopathological changes in the Brain of Gold fish *Carassius auratus*

The Photomicrograph brain of Control Group I Gold fish showed normal neuronal cells. No vacuole was observed. The photomicrograph of brain exposed to 10mg/l concentration of zinc sulphate Group II Gold fish exhibited vacuolar changes with empty spaces which appeared as moth eaten area. Degeneration of neurons and necrosis was also noticed. The photomicrograph of brain exposed to 15mg/l concentration of zinc sulphate treated Group III Gold fish showed vacuolation and degenerating of neurons with binucleated nuclei. These similar findings were observed by Omitoyin *et al*., (2006).



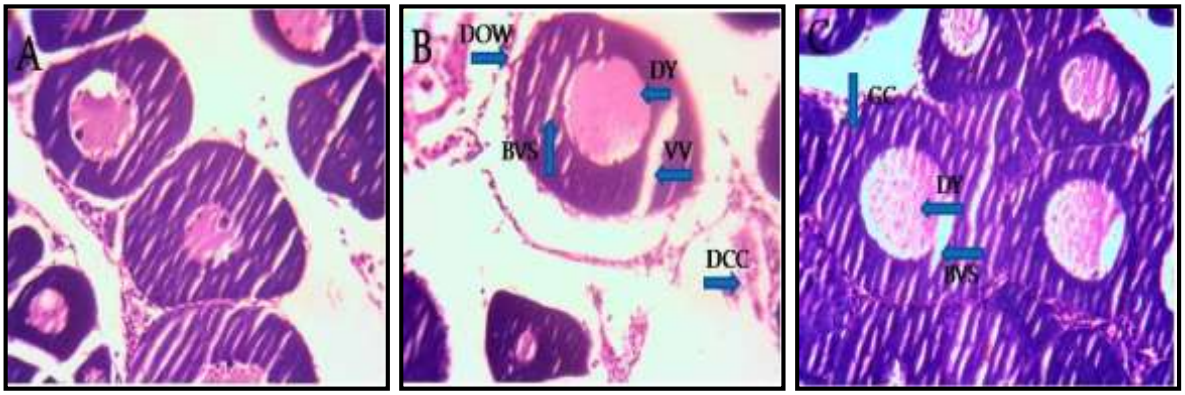
(a) Group I **(b) Group II** **(c) Group III**

Fig. 4: hotomicrograph shows Brain of Gold fish *Carassius auratus* (400x). (a) Group I – control; (b) Group II – 10 mg/l conc. of Zinc sulphate; (c) Group III – 15 mg/l conc. of Zinc sulphate. Binucleated nuclei (BN); Necrosis (N); Vacuolation (V); Degenerating neurons (DN).

3.4 Histopathological changes in the Ovary of Gold fish *Carassius auratus*

The ovaries of Control Group I Gold fish showed normal oocytes with mature and immature stages. Well-developed yolk and nucleus were noticed. The photomicrograph of histopathological examination of ovaries revealed that the fishes exposed to 10mg/l concentration of zinc sulphate Group II showed disintegrated columnar cells, vacuolated vitellogenic substances, numerous breaks in vitellogenic substances. Disintegrated ovarian wall and disappearance of yolk were also noticed. The photomicrograph of ovary at 15mg/l concentration of zinc sulphate treated Group III Gold fish showed severe damage of oocytes. Degeneration of yolk and breakage in vitellogenic substances clumping of globulin were also noticed. The photomicrograph of different organs of Gold fish *Carassius auratus* revealed the histopathological effect of zinc sulphate.

The damages of the organs have increased at higher concentration. Fishes exposed to 10mg/l concentration of zinc sulphate showed damage of gills, liver, brain and ovary. At 15 mg/l zinc sulphate concentration more vacuolation, necrosis and degeneration were observed. The same finding were observed by Sharma *et al*., (2011) and have suggested that long term exposure of heavy metals have resulted in marked degenerative changes in ovary.



(a) Group I **(b) Group II** **(c) Group III**

Fig. 5: Photomicrograph shows Ovary of Gold fish *Carassius auratus* (400x). (a) Group I – control; (b) Group II – 10 mg/l conc. of Zinc sulphate; (c) Group III – 15 mg/l conc. of Zinc sulphate. Disintegrated ovarian wall (DOW); Vacuolated vitellogenic substances (VV); Disappearance of yolk (DY); Break in vitellogenic substances (BVS); Disinfected columnar cells (DCC); Globulin clumping(GC).

Histopathology of all the organs showed more damages and the damage increased with increase of zinc sulphate exposure. The results, revealed increase in concentration is directly proportional to the increase in damages to the organs. In case of organs, the gills got severely damaged when compared to other organs. This may be due to direct exposure of gills to the zinc sulphate. The histopathological studies revealed the damage caused due to zinc sulphate in various organs of Gold fish have been in this hierarchy.

IV. CONCLUSION

In general, zinc sulphate creates manifold disturbances in the target tissues. Fishes are therefore particularly sensitive to environmental contamination of the water and pollutants like heavy metal may cause significant impairments of certain physiological and biochemical processes which can result in serious tissue damage. Fishes being important food resources affects aquatic ecosystem and enters human systems through food may lead to many serious problems. It is very important that zinc toxicity affects immune function changes and adverse effect on human body. Heavy metal toxicity has to controlled since it affects the aquatic ecosystem and turn other natural resources in turn causes toxicity to plants, animals and humans.

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