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Toxicity Effect of Cadmium Chloride on the Some Organ Tissues of Fresh Water Fish *Ophiocephalus* (Channa) *Striatus*

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Abstract

The aquatic Medium is an very diverse and highly fluctuating system. It is very important for the survival of life on earth. The physico-chemical properties of natural water is different in different areas. Metal pollution in water is in large part due to agricultural run-off, industrial waste & mining activities. So in the present investigation to show the effect of Cadmium chloride on the liver and pancreas of the freshwater fish Channa stiatus. The pancreas is the second largest digestive gland in the body of fishes. It comprises two histologically & functionally different components, an endocrine portion that secrete hormones and an exocrine portion secreting the digestive pancreatic juice. The endocrine portion of pancreas related to secreting hormone insulin and glucagon. Both are maintains the blood glucose level. Ilets of Langarhans of pancreas plays an important role in carbohydrate metabolism. In the present investigation control fish shows normal hepatic tissue hepatocytes with granular cytoplasm with round nucleus hepatocytes cells is observed in liver where as in experimental (LC_{50}) at 96 hrs.shows degenerative changes in hepato-cellular dissociation necrosis and hypertrophy is observed at 96 hrs. (LC_{50}) liver of fish.

In the present investigation in pancreas in Control fish normal structure of pancreas with pancreatic blood capillary is observed where as in experimental (LC_{50}) at 96 hrs. Pancreas shows Congestion hyperplasia, edema, & necrosis is observed. So that histopathological study of liver and pancreas is important tool for diagnosing the assessment of carbohydrate metabolism of the organisms.

Key wards: *Cadmium Chloride, LC_{50}, Liver, Pancreas, Histopathology, Channa striatus, Metabolism*

Introduction

Earth crust are made up of different metals and humans are come in contact with many different metals from the early phases of human civilization. The present rate of exploitation, direct and indirect use and concentration of heavy metals are in the environment is very high compare with previous decades. As environment point of view heavy metals are very complex. The trace amount of some of them are essential for plants and animals life. Probably the oldest toxins recognized by our species were probably derived from food from the plants and meat. Probably the metals were the first industrial toxins. Heavy metals like cadmium chloride, mercury chloride, Lead acetate which are present in industrial waste products and it is create serious water pollution problem. It was detected that cadmium content in tissues of fish living in Minamata Bay in Japan is very high (Thomas 2006). Eating of these cadmium contaminated fish in Japan came to public attention in 1956 when 121 Peoples are died due to eating contaminated fish. Thomas (2006) reported that the symptoms of lack of coordination paralysis, difficulty swallowing, convulsions & brain damage became known as Minamata disease.

Environmental pollution by heavy metals is very prominent in areas of mining & old mines sites & pollution reduces with increasing distance away from mining sites (Peplow 1999). Through mining activities water bodies are mostly polluted (Garba 1995) through rivers & streams, the metals are transported as either dissolved species in water or as an integral part of suspended sediments. Increase the heavy metals concentration in aquatic ecosystem due to the natural, geochemical and anthropogenic factor, the infiltration. These toxic heavy metals in aquatic ecosystem are carried via the food chain to the upper trophic level & create a serious ecological problem. (Bedi Cicik & Kenan Engine 2003)

United State Environmental protection agency (U.S.E.P.A.) listed 24 extremely hazardous substances. These include heavy metals also. Heavy metals and their salts constitute a very important group of environmental pollutant, since they are potent metabolic inhibitor. Due to differences in the area's climate, geomorphology, geology, and type of soil the physico-chemical properties of natural water is diverse in different areas of the world (Davis & Day 1998).

However, slow & continuous changes by man over the ages have meant that the vast majority of waters on the earth's surface may no longer be regarded as being in their natural state with respect to their dissolved & particulate matter (Jobling 1995 Most importantly the mining of metal ores & coal produced contaminated drainage water, resulting in increased heavy metal concentrations in flowing & standing waters (Llyod 1992).

Heavy metals belong to a group of elements whose cycles have been greatly influenced by man (Landis & Yu, 1995). Virtually all metals are toxic to aquatic organisms & because of the devasting effect of these metals on humans, heavy metals are one of the most toxic forms of aquatic pollution (Laws 2000). While some metals such as iron & copper may be regarded as micronutrients & needed in small concentration, others such as cadmium (Cd) & arsenic (As) are not needed (Laws 2000). Aquatic organism shows adverse effects due to accumulation of trace amount of these elements and it will be detrimental to aquatic organisms. Metal concentration in the aquatic medium increases due to a variety of causes like mining drainage water, effluent from the tailing ponds and drainage water from different metal industries continue to extrude unwanted metals in to the aquatic environment (Lloyd 1992).

Materials & Methods

All the fishes were dissected out on the 4th day of experiment (96 hrs.) at median lethal (LC_{50}) concentration of cadmium chloride along with control. Pancreas of experimental (LC_{50}) & control at 96 hrs. Fishes were separated and immediately transferred into 10% buffered formaldehyde solution. Tissues or organs for histological examination require appropriate processing, so that they are imparted optimum hardness for section with a microtone.

The important areas of operation covered are as follows:-

1. **Trimming and capsule filling:**

Done after 48hrs. Preserved in buffered formalin solution.

2. **Processing of tissues (washing, dehydration cleaning, and paraffin infiltration) :**

The foundation of good histological preparation requires adequate and complete fixation. Each organs transverse section were taken & used for washing. Washing of tissues has done for 4 to 5 hrs. under slow running tap water. Dehydration done by using as following alcohol treatment.

Alcohol (80%) for 1.5 hr. → Alcohol (95%) for 1.5 hrs. → Absolute alcohol for 1.5 hrs. → absolute alcohol for 2 to 3 hrs all the tissues was processed by using xylene and transferred to molten paraffin wax (58-60^0 C). Then all the prepared blocks was used for tissue holding section and it was done at 3 micron rotatory microscope-after staining. Microphotographs were taken in Image-R- Microscope camera.

3. **Staining and mounting:**

All the nuclei shown in blue color due to haematoxylin and eosin staining and rest of tissue parts were identifying by pink color staining.

4. **Mounting of tissues:**

All the tissue of liver and pancreas are fixed on slide with paraffin wax and covered with cover slip and this slide was examined under microscope.

5. **Microscopic examination:**

Use 7 megapixel digital Panasonic cameras for observing all the tissues. All the slides were observed under low and high resolution for their histological peculiarities.

Results:

In control liver Showing granular cytoplasm with rounded unclear hepatic cells (Fig-1) where as in experimental (LC_{50}) at 96 hrs. hepato-cellular dissociation, necrosis & hypertrophy is observed in liver of fish (Fig – 2).

In present investigation Control fish normal structure of pancreas with pancreatic blood capillary is observed (fig-3) where as in experimental (LC50) at 96 hrs. Congestion hyperplasia, edema, & necrosis is observed (fig-4).

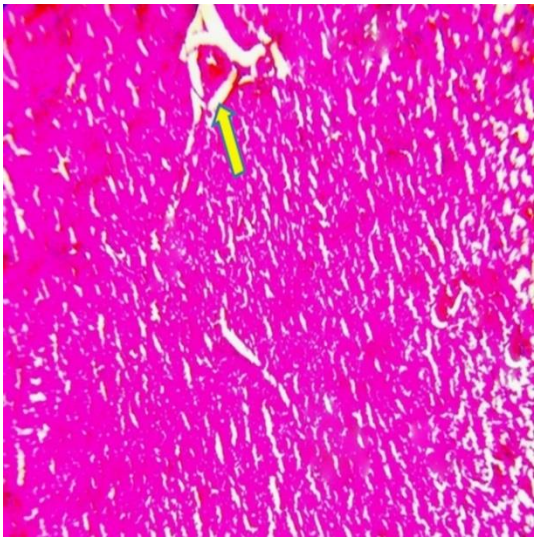

Fig- 1 Showing Microphotograph of Ophiocephalus (Channa) striatus control pancreas shows normal structure of pancreatica blood capalliery (Yellow arrow)

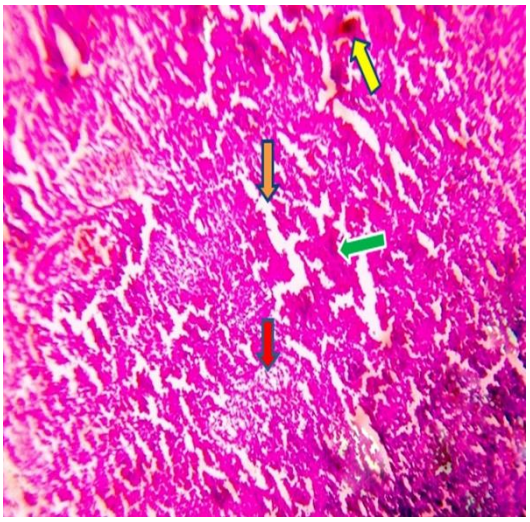

Fig- 2 Showing Microphotograph of Ophiocephalus (Channa) striatus Experimental pancreas shows congestion (Yellow arrow) hyperplasia (red arrow) edema (green arrow) and necrosis (orange arrow)

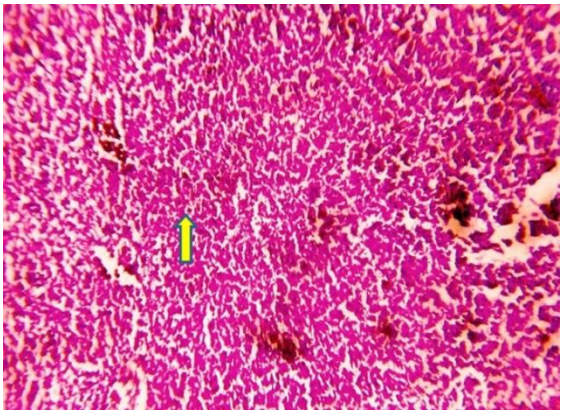

Fig- 3- Showing Microphotograph of Ophiocephalus (Channa) striatus control pancreas shows normal Hepatic tissue Hepatocyte with granular cytoplasm with round nucleus hepatocytes cells (yellow arrow).

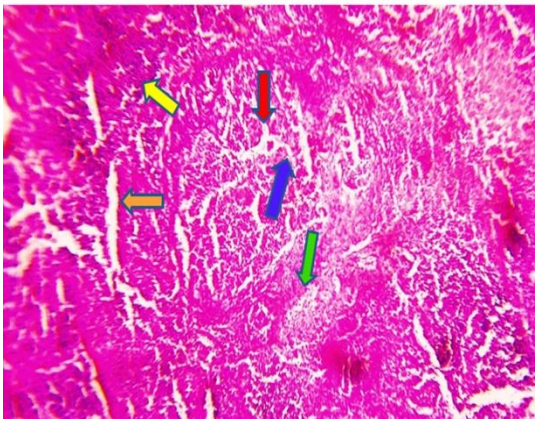

Fig- 4 Showing Microphotograph of Ophiocephalus (Channa) striatus Experimental pancreas shows hepatocellular dissociation (green arrow) degenerative includes cloudy swelling (yellow arrow) hypertrophy (blue arrow) and necrosis (orange arrow)

Discussion:

Benedetti et al (1989) studied on the high concentration of copper pollution (5ppm) and low concentration (0.3ppm) on bullhgeads *inctaturus nebulosus* and observed that the liver exposed at either concentration did not reveal diffuse changes in the hepatic parenchyma. They reported that areas of the cells aggregation on hepatic degeneration. Further they working on histochemical staining demonstrated lower liver glycogen contents than in the controls in all treatments with scattered areas of normal & depleted glycogen content in the treated specimen. Khalidah S. et.al (2010) studied on histopathological changes in the intestine, liver and pancreas of the common carp, Cyprinus carpio, during starvation and observed that the anterior intestine, no characteristic pattern of increase or decrease in the height of the mucosal cells was seen between the non starved and starved groups within eight weeks and histological observations of pancreas and proteolysis of intestinal epithelium.

In present investigation decrease glycogen in liver is possibly due to the degenerative changes in experimental (LC50) at 96 hrs. liver of fish. Similarly histopathology study in snake head fish *Channa punctatus* to the effects of 6.8 mg/L lead nitrate for 125 days showed liver cord disarry necrosis inflammation of portal areas hardening of connective tissue shrinkage of nucleus & septa formation around blood vessels (Sastry et.al., 1978b) No fatty infiltration or glycogen depletion was observed Treatment of snake head fish *Channa punctuates* with a sub-lethal concentration of lead nitrate produced considerable hepatic structural damage & decreased alkaline phosphate & aminopeptidase's activities.

Sorensen (1976) studied on Ultrastructural changes in the hepatocytes of green sunfish, *Lepomis Cyanellus* exposed to solutions of sodium arsenate and observed that the arsenic concentrations at 0, 30 or 60 ppm shows rate of change in hepatocyre ultrastructural morphology and appearance of electron dense particle cytoplasmic & intranuclear & cytoplasmic electron dense particle (EDP) exposed in 2 weeks. A series of experiments by Sastry &

Gupta (1978 a) working on effect of mercuric chloride in the liver of *Channa punctatus* for a period of 30 days and reported that hepatocellular granulation & vacuolation of the cytoplasm hypertrophy of nucleus necrosis, fatty infiltration, proliferation of connective tissue, glycogen depletion & cirrhosis. In the present investigation similar changes are observed like hapatocellular dissociation which leads to the complete dissolution of hepatocytes. Hepatocytes can contains glycogen & lipids which are related to the normal metabolic function of the liver with biochemical changes observed significantly depleted both in glycogen & lipid. So decreased glycogen & lipid in liver is caused due to the histological changes in experimental liver of fish. Cloudy swelling is characterized by the enlarged cell size possibly due to the endocrine dysfunction leads to the increase in transaminase activities. In present work of haematological studies SGPT & SGOT level is increased significantly in experimental fish over the control. So that it is also evident that liver dysfunction also responsible for increased SGPT & SGOT level in experimental haematological studies.

Dixon & Leduc (1981) studied the chronic cyanide poisoning of rainbow trout & its effects on growth respiration, & liver histopathology. Juvenile rainbow trout *Salmo gairdneri* exposed to concentration of 0.01, 0.02 & 0.03 mg/lit. hydrogen cyanide for 18 days showed widespread degemrative necrosis of hepatocytes at all concentration tested.

Ummi Fahmi et.al (2019) worked on histopathology of liver and intestine of pangkilan bare fish (Oryzias matanensis) Polluted by nickel and iron in Lake Matano, South Sulawesi and observed that the changes occurs in the liver and intestine in the form of hemorrhage, necrosis, fat degeneration, hydropic degeneration. Tridayani and Gusti (2010) Working on pengaruh logam timbal (pb) terhadap jaringan hati ikan kerapu bebek (Cromileptes altivelis) Maspari observed that the hemorrhage or bleeding is characterized by the presence of blood spots in blood vessels.

Abdus and Badrunesa (2001) mentioned the rupture of blood vessel pyknosis in necrosis and vacuolation is spotted murred *Channa punctatus* exposed to 100 ppm of sumition exposed liver of fish.

Gutierrez et.al., (1978), Tafanelli & summerfelt (1975) mentioned the hepatic lesions of fatty infiltration nuclear or general hypertrophy of hepetocytes other degenerative changes in paraenchyma loss of hepatic glycogen coagulative hepatic necrosis in *halobatracus didactylus* & *goldfish* expose to cadmium.

Sorensen et.al. (1983a) mentioned the hypertrophy & hyperplasia of exocrine pancreas in selenium exposed fish. In present work normal structure of pancreas with normal blood capillary (fig-1) is observed in control fishes where as in experimental ((LC50) at 96 hrs shows congestion which is characterized by excessive amount of blood within the vessels causes usually due to insufficient venous drainage of blood. This congestion is possibly resulting due to the alteration or problem in normal respiration along with damaged respiratory organs. In present work experimental gills of fish is extremely damaged (fig-2) possibly for this reason insufficient availability of oxygen causes the insufficient venous blood drainage.

Also in present work hyperplasia is observed in experimental (LC50) at 96 hrs. Pancreas of fish which is characterized by increased proliferation of a cell population usually in response to irritant (toxic or infections) & endocrine imbalance. In present work of biochemical & hematological studies glucose level in tissue & serum of experimental fish increased over the control which possibly due to the endocrine dysfunction along with hyperplasia of pancreas in experimental (LC50) at 96 hrs fish.

In present work edema & necrosis is also observed in experimental fish (fig-3). Edema is characterized by extracellular fluid resulting in a jelly like appearance with excessive amount of water collecting in intercellular spaces. This condition appeare possibly due to the alteration in water circulation within the body. Necrosis is characterized by the rapid & complete dissolution of cells & tissues leaving ragged-edges possibly caused due to the cadmium toxicity. Schulz (1971) reported the islet cell hyperplasia, subcapsular & aciner cell necrosis, vascular congestion, infraction & edema in pancreas of *Cyprinus carpio* caused by sub-lethal concentration of herbicides (dowpon).Eller (1971) mentioned the edema, congestion islet hyperplasia & necrosis in pancreas of *Cutthroat trout* chronically exposed to insecticides endrin.

Dimichele and Taylor (1978) reported that islet cell hyperplasia aciner cell necrosis, vascular congestion & edema in pancreas of *fundulus heteroclitus* exposed chronically to petroleum compound naphthalene. Above similar kinds of result in case of pancreas of different fish was mentioned by Couch (1975), Beville et.al. (1968).

Conclusion:

In present investigation liver of fish observed dissociation in hepatic cells which leads to the complete dissolution of hepatocytes. In experimental (LC_{50}) at 96 hrs. liver of fish decreased liver glycogen & lipid is caused due to the histological changes in experimental liver of fish. Unclear swelling is characterized by the enlarged cell size possibly due to the endocrine dysfunction leads to the increase in transaminase activities.

As the pancreas is dual organ having endocrine as well as exocrine in function so that any changes in pancreas leads to the alteration in normal metabolism of the fish. The condition of hyperplasia (An increased proliferation of cell population) possibly caused the hyper secretion of pancreatic enzymes carboxypeptidase's & nucleases. Hyperplasia condition is usually observed in response to irritant (toxic or infectious) endocrine imbalance. Hyperplasia possibly caused the increased serum glucose under the stress (experimental) condition for fulfilling the extra energy demand So that it is evident that altered pancreatic condition in experimental fish possibly the reason for several changes in biochemical as well as hematological studies in present work.

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Conflicts of Interest
The authors declare that there are no conflicts of interest regarding the publication of this paper.

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