



Effect of Mercury Chloride Induced Toxicity on Histology of Liver in in the Freshwater Fish *Hypphthalmichthys molitrix* (Valenciennes)

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KEYWORDS

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ABSTRACT:

Introduction: Anthropogenic activities are the major contributors to environmental pollution. Industrial discharges, automobiles, and roadways release particulate matter containing heavy metals such as mercury, cadmium, lead, arsenic, and chromium. These heavy metals enter the food chain through plant uptake, posing serious threats to human and ecosystem health. In aquatic environments, agricultural runoff containing heavy metals can be toxic to both plants and animals. Mercury, in particular, is known to cause neurotoxicity, nephrotoxicity, and hepatotoxicity in humans.

Objectives: The present study aimed to examine the effect of sublethal concentrations of mercury chloride on liver histology in the freshwater fish *Hypphthalmichthys molitrix* (silver carp).

Methods: Freshwater fish *Hypphthalmichthys molitrix* were exposed to sublethal concentrations of mercury chloride for durations of 10, 20, and 30 days. Liver tissues were dissected and processed for histopathological examination to assess mercury-induced alterations.

Results: Fish exposed to mercury chloride showed marked histological alterations in the liver, including vacuolization, necrosis, nuclear aggregation, enlargement of hepatocytes, and space formation. The severity of these changes increased with the duration of exposure, with the most pronounced alterations observed after 30 days.

Conclusions: The study concludes that mercury chloride induces significant histopathological changes in the liver of *Hypphthalmichthys molitrix*. Therefore, liver histology in this species can serve as a sensitive biomarker for assessing mercury pollution in aquatic environments.

1. Introduction

Globally, the Industrial Revolution played a significant impact on the development of the economies of many different countries because it changed an economy that was predominately based on agriculture and handicrafts into one that was dominated by industry and machine manufacturing. As a result, it led to unprecedented outcomes due to human activities and its negative impact on the environment. Their effects were environmental pollution that was formally categorized as anthropogenic activities, resulting from the dumping of industrial wastes, home trash and synthetic fertilizers and pesticides inputs. Therefore, to sustain the environment, alternative methods need to be employed

to mitigate the effects induced by manmade chemicals on the environment [1].

The aquatic environment with its water quality is considered the main factor controlling the state of health and disease in both cultured and wild fishes. Pollution of the aquatic environment by inorganic and organic chemicals is a major factor posing serious threat to the survival of aquatic organisms including fish [2]. Heavy metals are toxic metals that are dangerous when entering the body beyond the threshold. Heavy metal is an industrial waste that threatens aquatic organisms. Metal compounds can enter very easily and quickly into the body and can accumulate in the body tissues of aquatic organisms. It can also be toxic to humans if they



consume contaminated fish. The process of accumulation of metals in tissues occurs after absorption of metals from water or through contaminated feed. The metal is absorbed by the blood, binding to blood proteins, which are then distributed to all body tissues. Heavy metal pollution has negative effects on the life of living things such as disrupting chemical reactions, inhibiting the absorption of essential nutrients [3].

Heavy metals have harmful effects on human health, and exposure to these metals has been increased by industrial and anthropogenic activities and modern industrialization. Contamination of water and air by toxic metals is an environmental concern and hundreds of millions of people are being affected around the world. Food contamination with heavy metals is another concern for human and animal health. Concentration of heavy metals in water resources, air, and food is assessed with this regard. Metals among the other environmental pollutants may also occur naturally and remain in the environment. Hence, human exposure to metals is inevitable [4]. Heavy metals may deposit in sediment by ion exchange reaction, precipitation, absorption and finally contamination in fish may cause a potential health risk to men since metals can bioaccumulate in the food chain [5].

Mercury (Hg) is a naturally occurring element found in rocks, soils, sediments and the atmosphere. It is a relatively stable metal that does not readily react chemically. Mercury will vaporize at relatively low temperatures and can enter the atmosphere through the combustion of mercury containing materials (e.g., coal) and through natural processes such as the eruption of volcanoes. Historically, mercury was used in many ways including the manufacturing of mirrors and hats, as a fungicide for agricultural applications, as an ingredient in paints, as a processing chemical for production of chlorine and as a component of electrical products such as batteries and fluorescent lamps. During wartime mercury was used as a component of detonators for ordinance. Over the years as the toxic potential of mercury has become better understood, many of these applications have been discontinued and the quantity in use today is significantly less than in the past. Mercury occurs naturally in coal and, when burned, the combusted coal releases mercury into the

atmosphere. Coal fired power plants are significant source atmospheric mercury globally. Mercury is emitted into the environment through three main processes. The first is a natural process: mercury emitted from soils, volcanoes, the weathering of rocks and forest fires. The second is the anthropogenic (man-made) process: mercury emitted as a result of human activities such as the burning of municipal waste and fossil fuels. The third process is when previously deposited mercury is re-emitted or recycled to the environment through various natural processes such as the cycling of mercury between the oceans and atmosphere. The original source of this recycled mercury can be both natural and anthropogenic.

The major pathway of mercury to fish is atmospheric deposition. Mercury is emitted to the atmosphere in one of three forms: gaseous elemental, gaseous divalent and particulate. Gaseous elemental mercury is relatively insoluble and can remain in the atmosphere for up to a year and can travel long distances. Gaseous divalent and particulate mercury tend to drop out of the atmosphere in relatively quickly limiting the distance it travels from its original source. Elemental mercury emitted to the atmosphere is deposited state wide and explains why even fish that reside in remote lakes and ponds contain some amount of mercury from manmade sources. Divalent and particulate mercury forms that are deposited on the landscape are likely to have originated from more local sources.

Mercury (Hg) is the most toxic heavy metal in the world. The fish received Hg mainly through contaminated food, which can be determined by fish size, diet, ecological parameters, and water quality parameters such as a rise in water temperatures attributed to climate change may stimulate the methylation of Hg. The major Hg toxicity occurred due to the organic form of methyl mercury (MeHg^+) and estimated that 70 to 100% of the Hg in fish is present as MeHg^+ . The methyl mercury has created pollution in Minimata Bay, Japan. The mercury and its compounds could be retained in the tissues of fish for long periods, resulting in irreversible damages, such as neurological impairment and lesions, behavioral and cognitive changes, ataxia, as well as convulsions, in addition to its harmful effect on reproduction [6].



Fish histopathology provides a sensitive indicator of pollutant induced stress due to the central role that the organs play in the transformation of different active chemical compounds in the aquatic environment particularly the gills, kidneys, and liver are considered key organs for toxicological studies [7]. *Hypophthalmichthys molitrix* is one of the exotic fish. It is an edible freshwater fish of great importance. Hence, it is prudent to study the effect of sublethal concentration of heavy metal, mercury chloride on histopathological alterations in liver.

2. Materials and Methods

Procurement and rearing of experimental fishes

Hypophthalmichthys molitrix exotic carp commonly known as silver carp is widely distributed in the freshwater. *Hypophthalmichthys molitrix* was collected from the fish farm located in Puthur, 10 km away from the University Campus. The collected fishes without least disturbance were transported in polythene bags filled half with water. About 100 fishes were put in each bag and water was well aerated, using pressurized air from a cylinder. This mode of transit proved successful, since there was no mortality in all consignments throughout the study.

The fishes brought to the laboratory were acclimatized in cement tank for a fortnight before they were used for the experiment. The fish tanks were kept free from microbial infection by washing with potassium permanganate solution. The fish were disinfected with 0.1% potassium permanganate solution and were maintained for three weeks in well aerated tap water. Test fishes were critically screened for the signs of disease, stress, physical damage and mortality. The injured, severely diseased, abnormal and dead fishes were discarded. Feeding was discontinued two days prior to the commencement of the experiments to reduce the additive effects of animal excreta in the test trough [8]. The fishes were exposed to sublethal concentration of mercury chloride and control for the period of 10, 20 and 30 days. A control group was maintained with identical environment. The toxicant water and normal water was renewed every day. The fish were sacrificed from both experimental and control groups on 10, 20 and 30 days of exposure periods.

Experimental Design

Hypophthalmichthys molitrix weighing 14-16 g were divided into 4 groups and stocked at random into 4 different group were assigned.

The following experimental groups were conducted in the freshwater fish *Hypophthalmichthys molitrix* for the period of 30 days.

Group I: The first group was maintained in free from mercury chloride and served as the control

Group II : Fish exposed to 1/15th of the LC₅₀ of low sublethal concentration of mercury chloride for the period of 10, 20 and 30 days.

Group III : Fish exposed to 1/10th of the LC₅₀ of medium sublethal concentration of mercury chloride for the period of 10, 20 and 30 days.

Group IV : Fish exposed to 1/5th of the LC₅₀ of high sublethal concentration of mercury chloride for the period of 10, 20 and 30 days.

Fishes were exposed to sublethal concentrations of mercury chloride separately in plastic troughs and control fishes were also maintained separately. They were fed on ad libitum diet of rice bran and oil cake. The medium was renewed daily with sublethal concentrations of the mercury chloride. After the exposure period, *Hypophthalmichthys molitrix* were sacrificed and the livers were removed for histopathological examination.

Histological studies

To examine the extent of cellular damage caused by mercury chloride the liver of the control and treated fish tissues were fixed in Bouin's fluid. After 24 hours, the standard histological technique was followed by the method of [9]. The tissues were dehydrated in ascending grades of alcohol. After dehydration in graded series of alcohol, they were cleared in acetone and xylol. Then, they are treated with cold and hot infiltration and embedded in paraffin wax (58°C - 60°C). Serial sections were cut at 6 to 8 μm thickness, deparaffinized in xylol, passed through descending grades of alcoholic series, hydrated in water, stained in Heidenhain's iron haematoxylin and counterstained with aqueous eosin. Stained sections were mounted in DPX for microscopic observations.



3. Results

Liver histology

The liver of control fish, *Hypophthalmichthys molitrix* comprised of a continuous mass of hepatocytes arranged in irregular cords. The hepatic cells were large, polygonal in shape with the nucleus almost centrally placed. Blood sinusoids were also seen among the hepatocytes (Fig. 1A to F).

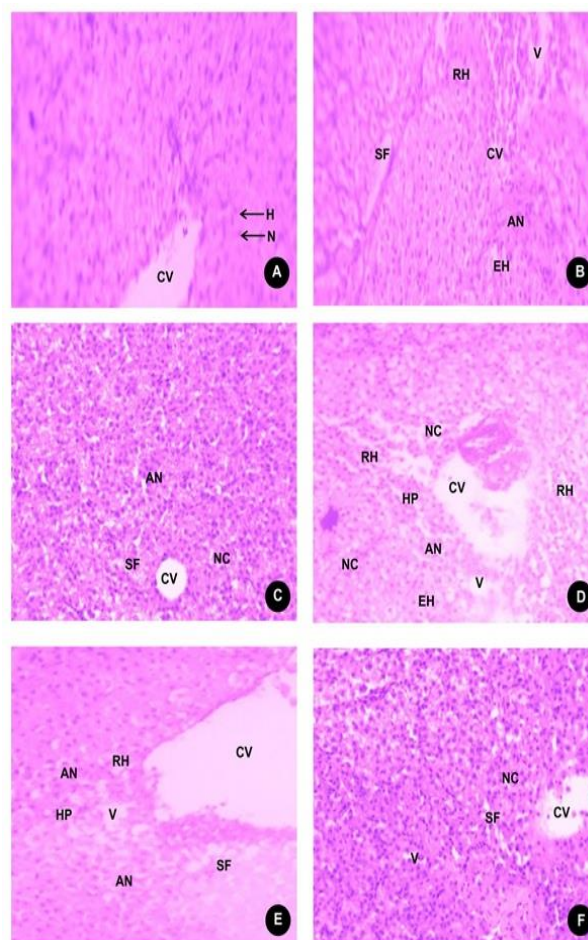
Liver histopathology

The structural organization of the liver appeared disturbed when fish were exposed to low, medium and high sublethal concentration of heavy metal mercury chloride for the period of 10, 20 and 30 days. Rupture of hepatocytes and their nuclei, necrosis, space formation and vacuolization of the liver, enlargement of hepatocytes, aggregation of nucleus and displacement of nuclei, hypertrophy were some of the observable changes. Additionally, disintegration of blood sinusoids and central vein were also observed in the liver. Severe changes were observed during 30 days exposure (Fig. G to J).

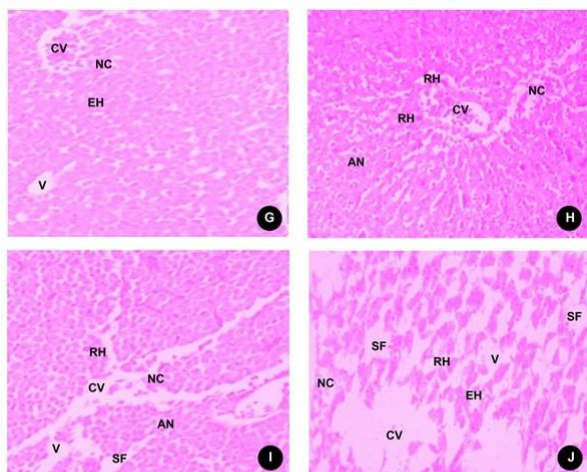
4. Discussion

Histopathology deals with the structure of the body tissue. Any unusual alteration

of cells can specify the effect of toxic substances and the presence of various diseases [10]. Liver is the most important centre for metabolising and also for detoxification. The liver has high concentration of xenobiotic metabolizing enzymes, some of which activate the toxicants to induce lesions locally [11]. In the present investigation rupture of hepatocytes and their nuclei, enlargement of hepatocytes, necrosis and vacuolization of the tissue, aggregation of nucleus and space formation are the important histopathological abnormalities observed in the liver of *Hypophthalmichthys molitrix* exposed to high sublethal concentration of mercury chloride for the periods of 10, 20 and 30 days. Similar changes in the liver of fishes have been reported in different species treated with various toxicants.



Focal coagulative necrosis and early cirrhosis has been reported in the liver of *Heteropneustes fossilis* exposed to sunset yellow and brilliant blue [12]. That presence of nuclear and cellular hypertrophy, irregular-shaped cells and nucleus, cellular atrophy, cytoplasmic vacuolation and eosinophilic granules in cytoplasm was common pathological characteristic of liver tissues of fishes exposed to heavy oil [13]. The liver of tilapia treated with cadmium showed degeneration of the hepatocytes with nuclear pyknosis, sinusoids with necrosed hepatocytes [14]. Dissociation of the hepatocytes and hydropic degeneration has been reported in the liver of *Cirrhinus mrigala* subjected to microcystin, LR, a cyclic peptide hepatotoxin produced by the cosmopolitan *Cyanobacterium microcystis aeruginosa* [12]. Accumulation of toxicants beyond a tolerable level in the liver may cause changes in the biochemical parameters and histological architecture [14].



A - Liver section taken from control fish showing normal histoarchitectural pattern (Xca 200)
 B - Liver section taken from low sublethal concentration of mercury chloride treated fish for the period of 10 days (XCa 200)
 C - Liver section taken from medium sublethal concentration of mercury chloride treated fish for the period of 10 days (XCa 200)
 D - Liver section taken from high sublethal concentration of mercury chloride treated fish for the period of 10 days (XCa 200)
 E - Liver section taken from low sublethal concentration of mercury chloride treated fish for the period of 20 days (XCa 200)
 F - Liver section taken from medium sublethal concentration of mercury chloride treated fish for the period of 20 days (XCa 200)
 G - Liver section taken from high sublethal concentration of mercury chloride treated fish for the period of 20 days (XCa 200)
 H - Liver section taken from low sublethal concentration of mercury chloride treated fish for the period of 30 days (XCa 200)
 I - Liver section taken from medium sublethal concentration of mercury chloride treated fish for the period of 30 days (XCa 200)
 J - Liver section taken from high sublethal concentration of mercury chloride treated fish for the period of 30 days (XCa 200)

CV - Central vein	NC - Necrosis
EH - Enlargement of hepatocytes	RH - Ruptured hepatocytes
H - Hepatocytes	SF - Space formation
N - Nucleus	V - Vacuolization
HP - Hyperplasia	AN - Aggregation of nucleus

Metal toxicity is not produced because of short-term exposure but continuous exposure even of small concentration for a long time is hazardous. Being axiomatic in its physiological status the histopathological changes of the liver due to toxic impact can be used as a reliable index to evaluate the structural and functional damage in animals [15].

Many histopathological studies indicated that liver is the organ to concentrate more pesticides and to show greatest damages or impairment. The severe destruction of hepatic cells and formation of intercellular spaces could possibly due to sudden withdrawal and utilization of stored glycogen from the liver cells to meet the energy demands during fungicide Cuman L. stress in *Sarotherodon mossambicus* [16].

The fish *Cirrhinus mrigala* exposed to zinc smelter effluent showed pycnosis

and cytolysis. Observed constriction of the bile duct lumen, pycnosis of nuclei, loss of glycogen and extensive degeneration of cytoplasm in *Heteropneustes fossilis* exposed to thiodon [17].

The breaking of the intercellular junction and necrosis were observed in the liver of *Heteropneustes fossilis* when exposed to hair dye [12]. In *Liza parsia* exposed to mercuric chloride and DDT caused severe liver damage due to hepatic lesions, destruction of cytoplasmic materials, vacuolization of the hepatocytes and blood sinusoids exhibited complete disorganization [18]. The liver filters the blood and supply throughout the body also play vital role in the metabolism [19]. The liver plays many roles in whole body function, such as the control and synthesis of critical blood constituents including glucose, free-fatty acids, ketone bodies, amino acids, hormones, clotting factors, and inflammatory mediators. The liver is critical in immune function and a first line of defense against certain infectious organisms and toxins entering from the gastrointestinal tract [20]. Histopathological examination of different sections of the *Catla catla* liver of treated fish showed degenerated hepatocytes, dilated sinusoids, vacuolar degeneration, eosinophilic infiltration, central vein contraction and necrosis [21]. The heavy metal exposed marine fish histopathology of liver showed that hemorrhage, degeneration of blood vessels, vacuolar degeneration, necrosis or cell death [22]. Degenerative changes in the liver of *Hypophthalmichthys molitrix* demonstrated the liver to be the organ affected most severely in response to mercury chloride intoxicification.

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