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Acute toxicity of Mercuric chloride on the survival and behavioural response of Indian fresh water stinging cat fish *Heteropneustes fossilis* (*Bloch*, 1794) at 48 and 96 hours

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ABSTRACT

The present investigation deals with the acute toxicity (LC_{50} evaluation) of mercuric chloride to Indian stinging cat fish *Heteropneustes fossilis* for 48 and 96 hours, the percentage of morality was calculated by the Finney's probit analysis method. The lc_{50} value of the prepared concentrations for 48 and 96 were found at 0.42 and 0.3 mg/l, respectively. During the course of experiment, the behavioural alterations were keenly observed like erratic movements, surfacing, increased mucous secretion, and change in body pigmentation, muscle fasciculation, loss of appetite, decreased opercular movements, ataxia, myotonia and uncoordinated movements. These altered behavioural changes were dose and time dependent. The present study also showed that the lc_{50} values gradually decreased with increase in exposure time and the mortality rate also increased with increase in toxicant concentration.

Key words : *Mercuric chloride, LC_{50}, Heteropneustes fossilis, Ataxia and appetite.*

Introduction

The Indian fresh water stinging Cat fish *Heteropneustes fossilis* (Bloch) is commercially one of the important high value native fish species (Rahman *et al*., 2013). It has both high market value and high consumer acceptance due to its high calcium, iron and omega 6 fatty acid content, less intramuscular spines, delicate taste and low omega 3 fatty acid. This native species is threatened nowadays due to poorly planned water management policy for irrigation, overexploitation, and also due to its changes in natural habitat because of rapid urbanization and industrialization (Chakraborty, 2010).

Mercury has been recognized among the most dangerous aquatic pollutant, due to its high toxicity, bioaccumulation, and bio-magnification properties in higher tropic levels and also due to its non biodegradable properties (Clarkson, 1990). Besides their toxic and harmful effects to aquatic life, heavy metals accumulate through the food chain and effects higher trophic levels including human beings too (Martin *et al.*, 2004). Mercury and its salts do not have any biological role but if present in any living organism it causes severe biochemical, haematological and histopathological alterations. The toxic effects of mercury mainly depend on its speciation form (oxidation state) and exposure pathway. The organic mercury and its vapor affects mainly central nervous system whereas mercuric chloride targets mainly the body's vital organs like kidney, liver and gastrointestinal tract (Vahter *et al.*, 2000; Ghosh *et al*., 2008). Fish absorbs heavy metal directly through contaminated water or indirectly from feeding on living organisms bio accumulated with heavy metal (Javed Md., 2015). Acute toxicity caused by different toxicant on fresh water fish can be evaluated by quantitative parameters like survival and mortality of test animals and sensitivity of different fish species against metal toxicity. Toxicity in fish is the cascade of events like physical, chemical and biological processes.

Lc_{50} is indicator of level of resistance of population response to metals (Reda *et al.*, 2010). The toxic pollutants effects water quality, feeding, swimming, body pigmentation and also delay for hatching on the maturation period (Atif *et al.,* 2005). Even though some heavy metals are essential for aquatic organisms in lower quantity for their normal physiological purposes, however, if present at high concentration levels, they accumulate in different organs depends on the type of heavy metal and their affinity with target organs, damages tissues, alters biochemical and hematological parameters, interferes with normal growth and proliferation (Atkarkhi *et al.*, 2009).

Mercuric chloride is used in agriculture as a fungicide, in medicine as a topical antiseptic and disinfectant and in chemistry as an intermediate in the synthesizing of other mercury compounds. The contamination of aquatic ecosystems by heavy metals due to anthropogenic and natural sources has gained increasing attention from the last three decades. The acute and chronic effects of heavy metals and pesticides to fish population have widely summarized by Jacob *et al.,* (1982). The present study is conducted to determine the lc_{50} value of mercuric chloride in *Heteropneustes fossilis* for 48 and 96 hours and to observe the behavioral responses in fish exposed to different concentrations of mercuric chloride.

Materials and Methods

Toxicant used: The toxicant used in this bioassay is inorganic mercuric chloride (CAS NO 7487-94-7), synthetic laboratory with 99% purity. A common stock of mercuric chloride solution was prepared by adding 5 g of mercuric chloride in 500 mL of double distilled water and the required quantity of mercuric chloride solution was drawn from the stock solution to maintain the desired concentration of toxicant in the experimental aquariums.

Experimental fish: The healthy and living Indian stinging cat fish *Heteropneustes fossilis* (Bloch, 1794) of 15-20 centimeters in length and 120-130 g in weight was used in five batches of bioassay. This species was selected for bioassay because it is easily raised under laboratory conditions; it fulfills the most of the requirements of a model species and is available throughout the year. These five batches of healthy and living fish were procured from local fish market in Indore, MP, India. These fishes were transported in plastic containers with utmost care to the laboratory. Before the start of acclimatization these were screened for any physical injury and dermal infections. Only healthy fishes were used in the whole study. They were disinfected with 0.2% of $KMNO_4$ prior to acclimatization for 2 minutes. These fish batches were acclimatized in 200 l container filled with de chlorinated water for 15 days under normal laboratory conditions. During this period they were fed with a dry commercial pelleted food (Taiyo Plus) with 25% of crude protein on alternate days. Water was changed gradually after every 24 hours to maintain the health status of water and afterwards, the fish were transferred to 50 l glass aquaria filled with de chlorinated tap water and covered with plastic film at the top to prevent evaporation. These glass aquaria were fitted with aerators for rich aeration. The experiments were conducted at a stock density of 10 fish per aquarium. Prior to start of experimentation physico-chemical parameters of water was determined (Table 1).

Experimental Design: The experimental design is based on the static renewal test (SRT), range finding and definitive test (Acute toxicity test) described by

Table 1. Physico-chemical analysis of water

S. No.	Water quality Parameters	Calculated Value
1	Temperature	32 ±4°C
2	pH value at 32 °C	7.42 ±0.2
3	Total Hardness as ($Caco_3$)	155 ±10 (mg/L)
4	Dissolved oxygen (mg/L)	7.6 ±0.5

Sprague and USEPA (1973). Basing on the pilot/ preliminary experiments, the experiments were conducted to determine the toxicity in different dose concentrations was selected. Five batches of acclimatized fish (10 fish in each batch) were exposed to 0.08, 0.16, 0.32, 0.38, 0.42 and 0.86 mg/l for 48 hours. The mortality and behavioral response was observed from 0.08 to 0.86 mg/l of mercuric chloride concentration. Later two batches of ten fish each were again exposed to concentration of 0.32 to 0.86 mg/l. The 50% mortality was recorded at the concentration of 0.42 mg/l for 48 hours and the confirmation of lc_{50} values for 48 hours was done with three replicates.

Similarly for the determination of the lc_{50} value at 96 hours, five acclimatized fish batch (10 in each batch) was exposed to 0.05, 0.10, 0.15, 0.20, 0.3 and 0.5 mg/l concentration of toxicant. The mortality and behavioral response was observed from 0.05 to 0.5 mg/l of mercuric chloride concentration. Later two batches of ten fish each were exposed to 0.2 to 0.5 mg/l. the lc_{50} value was recorded at 0.25 mg/l for 96 hours and the confirmation of lc_{50} at 96 hours was done with three replicates.

Results

Determination of median lethal concentration of mercuric chloride in the *Heteropneustes fossilis* for 48 and 96 hours (lc_{50} for 48 and 96 hours).

The lc_{50} value was calculated as the concentration of the test chemical which causes 50% mortality of fish during the respective periods of exposure i.e. 48 and 96 hours. Then the value of mercuric chloride value evaluates and found to be 0.42 and 0.25 mg/l for 48 and 96 hours respectively as shown in Fig 1.

The mortality was subjected to probit analysis and graphs were plotted between concentration of mercuric chloride and percent mortality of fish and between $\log_{10}$ concentrations versus probit kill. The percentage mortality was gradually increased with increase in concentration of heavy metal Fig 2.

During the experiment lc_{50} values are reduced and the exposure time is increased along with log concentration from 48 to 96 hours and no mortality was observed in control group.

Behavioral response of fish due to acute toxicity of mercuric chloride at different concentration

Behavioral changes seem to be most important indications of potent toxic effects because any behavioral alteration can be related to physiological marker in aqueous species (Kristiansen *et al*., 2009). During the course of experiment fish have exhibited different behavioral changes to the mercuric chloride concentrations which were dose and time dependent. Initially fish was alert and stopped swimming and remained in static position in response to abrupt changes in the surrounding environment. After some time they turned to avoid the toxic medium with fast swimming, jumping and hitting the walls of container. Then the fish becomes slowly restless and excessive mucous was uniformly secreted all over the body surface. They came to surface for gulping of air, defecation was increased and fecal matter was found at the bottom of container than the control fish group. The barbels of the fishes in experimental aquariums show pathological changes, with their external surface becoming white after some time of mercuric chloride exposure fol-

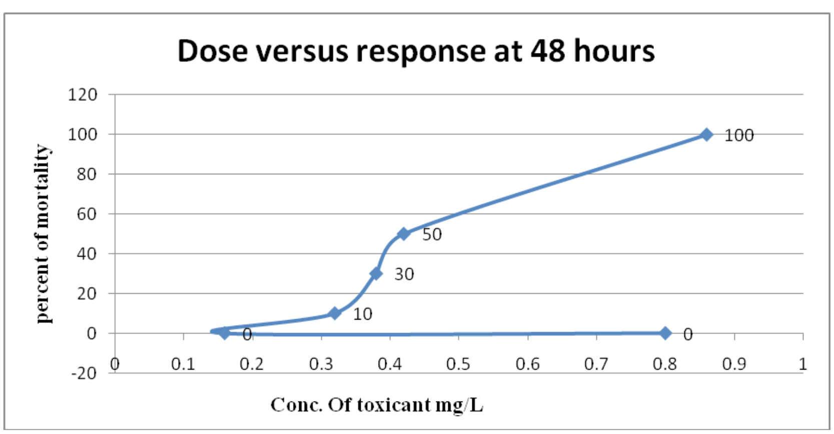

Fig. 1. Relationship between the concentration of mercuric chloride and mortality of Heteropneustes fossilis for 48 hours.

lowed by erosion of external epidermal lining and subsequent breaking off from the base leaving behind only stumps. Four days of mercuric chloride treatment at its lc_{50} and above concentrations causes hemorrhages and ulcers at many places on the lateral sides of the body in the form of blotches. In aquaria with high concentration of toxicant, the fish swim erratically with jerky movements and hyper excitability. Their fins become hard and stretched due to stretching of body muscles. Body pigmenta-

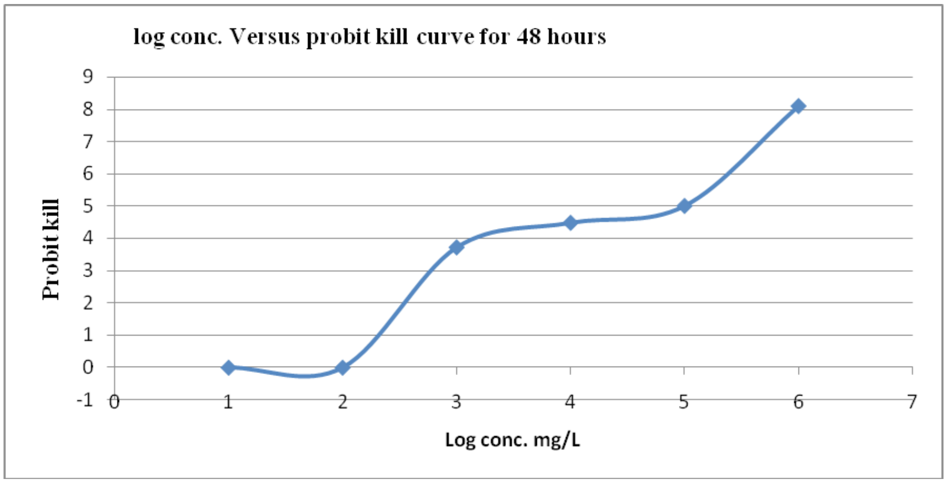

Fig. 2. Relationship between $\log_{10}$ concentration of mercuric chloride and probit of kill for *Heteropneustes fossilis* 48 hours

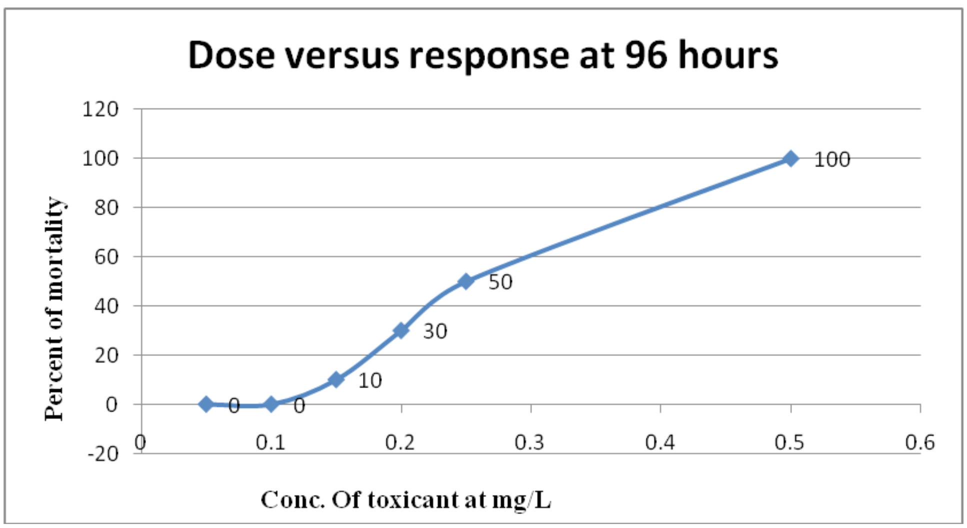

Fig. 3. Relationship between the conc. of mercuric chloride and mortality of *Heteropneustes fossilis* for 96 hours.

Table 2. Effect of mercuric chloride on the survival of *Heteropneustes fossilis* at 48 hours

S. No.	Conc. of toxicant mg/l	Percent of mortality	Log conc.	No. of dead fish	No. of exposed fish	Probit mortality
1	0.8	0	-0.096910013	0	10	0
2	0.16	0	-0.795880017	0	10	0
3	0.32	10	-0.494850022	1	10	3.72
4	0.38	30	-0.420216403	3	10	4.48
5	0.42	50	-0.37675071	5	10	5
6	0.86	100	-0.065501549	10	10	8.09

tion was decreased. Finally fish was swimming at the bottom of container due to ataxia, showing the signs of myotonia and aneroxia, become exhausted, do not gave response to stimulus, lose consciousness, and become lethargic with ventral side facing upward due to mercuric chloride intoxication and finally half of the fish population died at the end of 48 and 96 hours respectively. The major cause of fish mortality may be due to damage of respiratory epithelium by oxygen deficiency during the formation of thick mucus film over the surface of body Das and Sahu, 2005). No fish died in the control group.

Discussion

Acute toxicity (96-h lc_{50} and lethal concentration) of metals varies significantly among themselves. It is observed some fishes are very sensitive towards the toxicity caused by one heavy metal and shows less sensitivity towards another equally toxic heavy metal at the same time (Das and Banerjee, 1980). The lc_{50} at 96 hours values for inorganic mercuric chloride reported by FAO/UNEP (1991) is 0.35 mg/l for cat fishes. Impact of mercuric chloride on the survival of *Heteropneustes fossilis* have been studied by Rajan and Banerjee in 1991. They calculated the lc_{50} at 96 hours for *Heteropneustes fossilis* equivalent to 0.3 mg/l. The study of Pandey *et al.* (2005) confirmed the 96 hour lc_{50} value of mercuric chloride for *Channa punctatus* as 1.20 mg/l, while Khangarot (2003) reported the 96 hour lc_{50} value of mercuric chloride on *C. marulius* as 0.314 mg/l. The 96 hour lc_{50} value of mercuric chloride for *Fundulus diaphanous*, *Lepanis gibbosus* and *Cyprinus carpio* were found to be 0.11, 0.3 and 0.18 mg/l respectively (Boening, 2000) and for *Raccus saxatalis* and *Salvelines fantanalis* were found to be 0.35, 0.09 and 0.9 mg/l (Pandey *et al.*, 2005). Mohammad *et al.* (2009) calculated the 96 hour lc_{50} value of 0.35 mg/l in *Gambusia holbrachi* and Shakoori *et al*. (1991) reported the 96 hour lc_{50} value of mercuric chloride as 0.084 mg/l in small sized *Ctenopharyngodon idella.*

The 96 hours lc_{50} value calculated in present study is 0 mg/l which is equal to the value calculated by Rajan and Banerjee in 1991 and slightly lesser than what Pandey *et al.* (2005) calculated in *Heteropneustes fossilis* in 2005, this may be due difference in water hardness and pH of water . Several

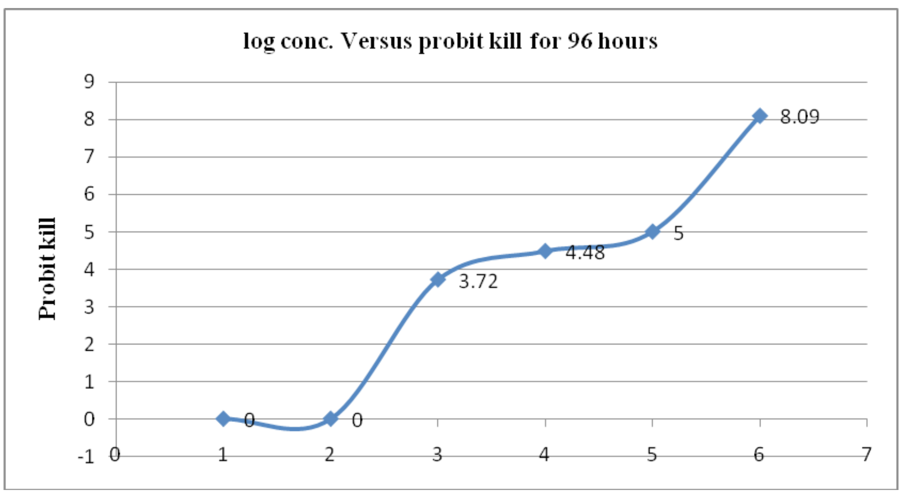

Fig. 4. Relationship between $\log_{10}$ concentration of mercuric chloride and probit of kill.

Table 3. Effect of mercuric chloride on the survival of *Heteropneustes fossilis* at 96 hours.

S. No.	Conc. of toxicant mg/L	Percent of mortality	Log conc.	No. of dead fish	No. of exposed fish	Probit mortality
1	0.05	0	-1.301029996	0	10	0
2	0.1	0	-1	0	10	0
3	0.15	10	-0.823908741	1	10	3.72
4	0.2	30	-0.698970004	3	10	4.48
5	0.25	50	-0.602059991	5	10	5
6	0.5	100	-0.301029996	10	10	8.09

physicochemical parameters of water affect the biological activity of heavy metal directly therefore making them less toxic. Water hardness and pH had a major role in heavy metal toxicity. Higher water hardness is beneficial in reducing metal toxicity to fishes (Weiner, 2000). Rathore and Khangarot (2003) found that the toxicity of mercuric chloride and water hardness is inversely proportional.

Results of static bioassays are still fragmentary and highly variable as the changes in size, species, chemical, biological and environmental factors influence the toxicity (Das and Sahu, 2005).

Comparison of current lc_{50} values with values calculated earlier may not meaningful because various factors may influence bioassay results like difference in test animal (species, weight and size and exposure route) and other environmental factors like pH, hardness and dissolved oxygen in test water.

Agarwal (1991) also reported similar altered behavior for *Channa punctatus* exposed to mercuric chloride, such abnormal and altered behavior considered to be as a result of excessive elimination of skeletal muscles (Pragatheeswarn *et al*., 1987), while excessive secretion of mucous over the body surface and dyspigmentation are attributed to the dysfunction of pituitary gland under the toxic stress, causing changes in number and area of mucous glands and chromatophores (Panday *et al*., 1990). The preceding abnormal behavior and subsequent death imply that the toxic effects are mediated through the disturbed nervous system enzyme system and vital cell membrane pumps affecting the physiology of fish.

Conclusion

Mercuric chloride, an inorganic salt of mercury is highly toxic even at low concentration (Lc_{50} 96 hours 0.3 mg/l) which causes large scale damage to different cellular components, biochemical parameters and hematological parameters of fish body. All the observations indicate the affect of mercuric chloride toxicity and caused abnormal behavior. Therefore the present study demonstrates a relation among heavy metals stress and survival and mortality rates. Therefore the present study can be used as a powerful tool for creating awareness among the local farmers and compare the sensitivity of various aquatic organisms and potential of effluent using lc_{50} values to drain safe concentrations so that the use of highly toxic metal can be minimized.

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