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Acute toxicity of mercuric chloride ($Hgcl_2$) on survival and behavioural responses of freshwater fish *Labeo rohita* (Hamilton)

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Abstract
The aim of the present study deals with the acute toxicity (LC_{50} evaluation) of Mercuric Chloride to fresh water fish *Labeo rohita* for 24, 48, 72 and 96 hours the percentage of mortality rate was calculated by following the Finney's probit analysis method. The LC_{50} values of the prepared concentrations for 24, 48, 72 and 96 hrs were found at 0.41, 0.35, 0.30, and 0.25 mg/L respectively. During the course of experiment, the behavioural alterations were also observed like surfacing, erratic movement, increased mucous secretion, decreased opercular movement and loss of balance. The present study revealed that, the LC_{50} value gradually decreased with the increase of exposure period and the mortality rate increased with increase in concentration with pesticide exposure.

Keywords: Mercuric chloride, LC_{50}, mortality, behavioural, *Labeo rohita*

1. Introduction

The heavy metals are important pollutants for fishes, because these are not eliminated from aquatic systems by natural methods, such as organic pollutants and enriched mineral organic substances. Mercury has been recognized as one of the most hazardous aquatic pollutants due to its toxicity, bio accumulative and non-biodegradable properties [1]. Besides their toxic and harmful effects to aquatic organisms, heavy metals accumulate through the food chain and may affect human well beings [2]. Some non-essential trace metals such as mercury, lead and cadmium are toxic at concentrations observed in natural waters [3]. Mercury and its compounds have not any role in biological functions but if present in any living organisms, it may cause cytochemical and histopathological effects. The organic mercuric compounds and mercuric vapour effects central nervous system. Whereas mercuric chloride targets liver, Gastro internal tract and kidney [4, 5]. Fish may absorb metal directly from contaminated water or indirectly from feeding on living organisms in the contaminated water [6, 2]. Although many research have been conducted to assess the toxicity of heavy metals in algae, however, the number of studies dealing with the toxic effect of heavy metal on aquatic animals including fish are limited [7]. The concentration of mercury in fish, even for humans consuming only small amounts of fish (10–20 g/day), can markedly affect the intake of methyl mercury [8]. Acute toxicity caused by different toxicant on freshwater fish can evaluate by quantitative parameters like survival and mortality of test animals and sensitivity of different fish species against metal toxicity. Toxicity in fish is the culmination of a series of events involving various physical, chemical and biological processes.

LC_{50} is indicator to the level of resistance of population response to metals [9]. The toxic pollutant effects water quality, feeding and swimming behaviour of fish and also delay the hatching, on the maturation period [10]. Even though some heavy metals are essential for aquatic animals in low concentrations. However, at high concentration levels, they accumulate in different organs, damage tissues and interfere with the normal growth and proliferation [11]. *Labeo rohita* is a commercial fish and widely preferred as edible fish in India and it is very important to evaluate edible organisms. The objective of the present work was to observe the behavioural changes and survival rates of fresh water fish *L. rohita* on exposure to mercuric chloride.

2. Materials and Methods
2.1 Fish
The fresh water fish, *Labeo rohita* (Rohu) (8-10 cm length and 28 ± 0.6 g weights) was used five batches of fish (10 fish in each batch) for the toxicity tests. These fishes were collected from local fish ponds at Nuzividu village in Guntur district in Andhra Pradesh, India (latitude: 16.1808576.N, longitude: 80.6646.E). The fishes were acclimatized to the laboratory conditions for 15 days. The tub containing fish was aerated with rich oxygen. The hygienic conditions were maintained by renewing water after every 24hrs and, fish were daily fed with rice bran and fish pellets.

Fig 1: *Labeo rohita*

2.2 Chemical
The inorganic Mercuric Chloride (HgCl2) (CAS number 7487-94-7), was used as the test chemical.

2.3 Acute exposure for Mercuric chloride
A common stock solution of mercuric chloride was prepared by dissolving 1 gram of pesticide in 100 mL double distil water and the required quantity of Mercuric chloride was drawn from the stock solution to maintain the standard concentration of 1mg/L in the container. The acclimatized fish were placed into separate container containing dechlorinated and aerated water. Basing on the pilot experiments, the experiments were conducted to determine the toxicity in different dose concentration was selected.Five batches of fish (10 fish in each batch) were exposed to 0.07, 0.14, 0.28, 0.34, and 0.41 mg/L for 24 hours. The mortality and behavioral responses of fish was observed from 0.28 to 0.41 mg/L of heavy metal concentration. Later, two batches of (10) fishes were again exposed to concentrations of 0.34 and 0.41 mg/L to evaluate the LC_{50} value for 24 hours. Then, the 50% of mortality was obtained at the concentration of 0.41 mg/L for 24hours and the confirmation of acute toxicity (LC_{50}) was performed with three replicates.
To assess the LC50 value for 48 hr, the fishes were exposed to 0.06, 0.12, 0.18, 0.28, and 0.35 mg/L concentrations of mercuric chloride. The fish mortality occurred from 0.12 to 0.35 mg/L concentration of Mercuric chloride. Then two batches of (10) fishes were exposed to the concentration of 0.28 and 0.35 mg/L. The 50% of mortality was occurred at the concentration of 0.35 mg/L for 48 hours and the confirmation of LC_{50} value was done with three replicates.
To evaluate 72 h LC_{50} value, the acclimatized fish were exposed to 0.06, 0.14, 0.24, 0.28 and 0.30 mg/L concentrations of mercuric chloride. The varied mortality of fish occurred from 0.14 to 0.30 mg/L concentration of toxicant. Then two batches of (10) fish were exposed to concentration of 0.28 and 0.30 mg/L. The 50% of mortality was obtained at the concentration of 0.30 mg/L and the confirmation of LC_{50} value for 72 h was done with three replicates. The LC_{50} for 96 h was found by exposing acclimatized fish, *L.rohita* to 0.05, 0.10, 0.15, 0.20 and 0.25 mg/L concentrations of heavy metal, mercuric chloride. The fish mortality began to occur from the concentration of 0.10 mg/L onwards. Then, three batches of 10 fish were exposed to the concentration of 0.15, 0.20 and 0.25 mg/L. The 50% of mortality was obtained at the concentration of 0.25 mg/L and confirmation of LC_{50} value for 96 h was done with three replicates.
Finally, the lethal concentration (LC_{50}) values of mercuric chloride exposure found to be 0.41, 0.35, 0.30, and 0.25 mg/L for 24, 48, 72 and 96 hr respectively. During this whole experiment a control group was maintained with normal to comparison. The percentage of mortality rate were calculated by following the probit analysis method (Finney OJ. 1971).The behavioral alterations in toxicant exposed fish were observed during the acute toxicity of Mercuric chloride.

3. Results
3.1 Determination of median lethal concentration (LC_{50})
The LC_{50} value was calculated as the concentration of test chemical which caused 50% mortality of total fish during the respective periods of exposure i.e., 24, 48, 72 and 96 hr. Then, the LC_{50} values of Mercuric chloride evaluated and found to be 0.41,0.35, 0.30 and 0.25 mg/L for 24, 48, 72 and 96 h respectively as shown in (Table 2-5).
The mortality data was subjected to probit analysis and graphs were plotted between concentrations of mercuric chloride and percent mortality of fish. The percent mortality was gradually increased with the increase in concentration of heavy metal (Figure 2-5).
During the experiment, the LC_{50} values are reduced and the exposure time is increased along with Log concentration from 24 to 96 h (Table 5) and no mortality was observed in control group.

Table 1: Estimation of physico-chemical parameters of water

Water Quality Parameter	Calculated Value
Temperature	27 ± 3^0 C
Turbidity	6.95 silica units
pH value at 28°C	7.32
Total Hardness as($CaCO_3$)	165 (mgl^{-1})
Total Suspended Solids (TSS)	5 (mgl^{-1})
Chemical Oxygen Demand (COD)	Nil
Biological Oxygen Demand (BOD)	7 -11ppm
Sulphates as (SO_4)	Trace amount
Phosphates	Trace amount
Dissolved Oxygen (DO)	5 - 7mgl^{-1}

Table 2: Effect of mercuric chloride on survival of *Labeo rohita* for 24hr

S. No.	Conc. of toxicant (mgl^{-1})	Log Conc.	No. of exposed fish	No. of dead fish	Percent of mortality	Probit Mortality
Control	-------		10	0	0	---
1	0.07	-1.154	10	0	0	--
2	0.14	-0.853	10	1	10	3.72
3	0.28	-0.552	10	2	20	4.16
4	0.34	-0.468	10	4	40	4.75
5*	0.41	-0.387	10	5	50	5.00
6	0.49	-0.309	10	6	60	5.25
7	0.57	-0.244	10	7	70	5.52
8	0.68	-0.167	10	8	80	5.84
9	0.72	-0.142	10	9	90	6.28
10	0.82	-0.086	10	10	100	8.09

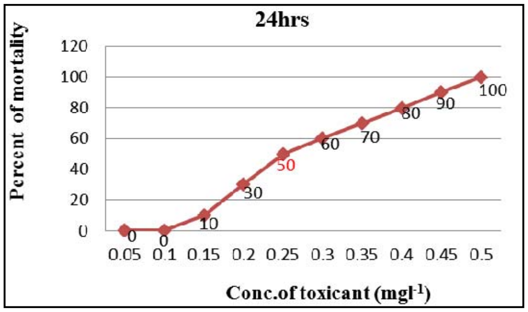

Fig 2: Relationship between the conc. of mercuric chloride and mortality of *Labeo rohita* for 24 hours

Table 3: Effect of mercuric chloride on survival of *Labeo rohita* for 48hr

S. No.	Conc. of toxicant (mgl^{-1})	Log Conc.	No. of exposed fish	No. of dead fish	Percent of Mortality	Probit Mortality
Control	-------		10	0	0	---
1	0.06	-1.221	10	0	0	---
2	0.12	-0.920	10	0	0	----
3	0.18	-0.744	10	2	20	4.16
4	0.28	-0.552	10	3	30	4.48
5*	0.35	-0.455	10	5	50	5.00
6	0.44	-0.356	10	6	60	5.25
7	0.55	-0.259	10	7	70	5.52
8	0.59	-0.229	10	8	80	5.84
9	0.65	-0.187	10	9	90	6.28
10	0.72	-0.142	10	10	100	8.09

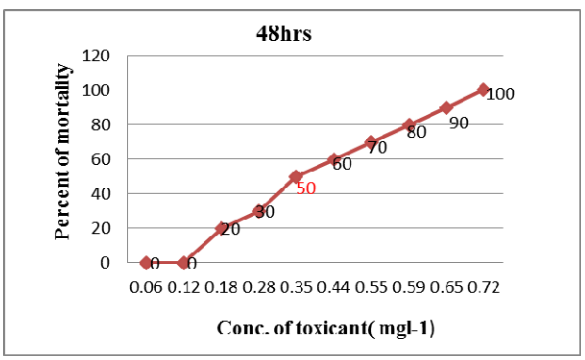

Fig 3: Relationship between the conc. of mercuric chloride and mortality of *Labeo rohita* for 48 hours

Table 4: Effect of mercuric chloride on survival of *Labeo rohita* for 72 hr

S. No.	Conc. of toxicant (mgl^{-1})	Log Conc.	No. of exposed fish	No. of dead fish	Percent of Mortality	Probit Mortality
Control	-------		10	0	0	---
1	0.06	-1.221	10	0	0	---
2	0.14	-0.853	10	2	20	4.16
3	0.24	-0.619	10	3	30	4.48
4	0.28	-0.552	10	4	40	4.75
5*	0.30	-0-522	10	5	50	5.00
6	0.35	-0.455	10	6	60	5.25
7	0.42	-0.376	10	7	70	5.52

8	0.54	-0.267	10	8	80	5.84
9	0.67	-0.173	10	9	90	6.28
10	0.76	-0.119	10	10	100	8.09

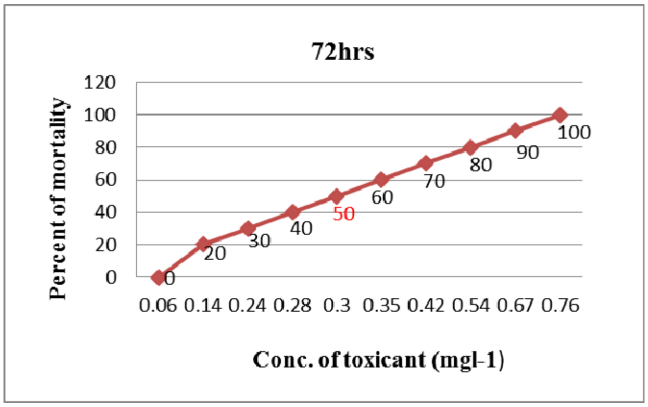

Fig 4: Relationship between the conc. of mercuric chloride and mortality of *Labeo rohita* for 72 hours

Table 5: Effect of mercuric chloride on survival of *Labeo rohita* for 96 hr

S. No.	Conc. of toxicant (mgl^{-1})	Log Conc.	No. of exposed fish	No. of dead fish	Percent of Mortality	Probit Mortality
Control	-------		10	0	0	---
1	0.05	-1.301	10	0	0	---
2	0.10	-1.000	10	0	0	
3	0.15	-0.823	10	1	10	3.72
4	0.20	-0.698	10	3	30	4.48
5*	0.25	-0.602	10	5	50	5.00
6	0.30	-0.522	10	6	60	5.25
7	0.35	-0.455	10	7	70	5.62
8	0.40	-0.397	10	8	80	5.84
9	0.45	-0.346	10	9	90	6.28
10	0.50	-0.301	10	10	100	8.09

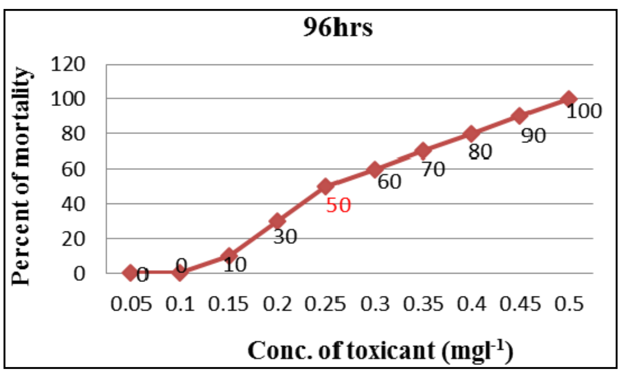

Fig 5: Relationship between the conc. of mercuric chloride and mortality of *Labeo rohita* for 96 hours

3.2 Behavioural changes in fish due to acute toxicity

Behavioural changes are the most sensitive indication of potential toxic effects. During the course of experiment, the fish have exhibited different behavioural changes to the Mercuric Chloride concentrations. In the initial stage, fish stopped swimming and remained in static position by sudden change in the surrounding water. Then rapid swimming with hyper excitation, jumping in the container at high concentration was noticed. Also vertical and downward swimming activities were observed followed by vertical hanging in the water and hitting to the container walls. Then the fish becomes slowly restless and excessive mucus was secretes from all over the body. Defecation was increased and more fecal matter was found at the bottom of the circular tubs than control group fish. Finally Fish was swimming at the bottom due to complete loss of balance and sank to the bottom with their ventral side facing upwards due to mercury stress.

4. Discussion

Acute toxicity (96-h LC_{50} and lethal concentrations) of metals varied significantly among fish species. Sometimes it is observed that 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 concentration [12]. Impact of different heavy metals and pesticides on the behaviour of *Labeo rohita* have been studied by various workers [13]. The findings of the present investigation mercury caused 100% mortality of *L. rohita* at 0.50 mg/L and 50% mortality at 0.25 mg/L during 96 h exposure and percent mortality was gradually increased with the increase in concentration of heavy metal mercuric chloride toxicity and behavioural alterations were also observed. Earlier studies revealed that the LC_{50} of a chemical for a species may vary under different environmental condition like time of exposure, size, and other impacts has reported the acute toxicity test was performed on Indian major carp *Labeo rohita* exposed to different concentrations of lead for 96 h the median lethal concentration (LC_{50}) was found to be 34.30 ppm [14]. Kousar S [15] has studied the effects of lead and arsenic accumulation and Bio concentration factor (BCF) in different organs at sub lethal concentration (33% 96 h LC50) were estimated that the caused mortality of four fishes the 96-h LC_{50} values of as were recorded as (Pb) 30.0±0.0, 24.5±0.1, 10.2±0.2 and 22.2±0.0 mg L-1, (As) 32.1±0.0,29.7±0.0 and 14.1±0.2 mg L-1 for *Labeo rohita*, *Cirrhinus mrigala*, *Catla*

catla and *Ctenopharyngodon idella*, respectively [15, 2]. Lethal concentration (LC_{50}) of aluminum chloride to *Labeo rohita* for 24, 48, 72 and 96 h of exposure are 44, 39.5, 36 and 32.5 ppm, respectively susceptibility of the aquatic biota to other chemical has been investigated by many workers [16]. The lethal concentration of 96h value of tannery industry effluent was reported as 28.32 ppm for *Labeo rohita* [17]. Rimon has reported the lethal concentration 96-h LC_{50} values of chromium were recorded 3.322 ppm for *Labeo* rohita [18]. Muthukumaravel reported the (LC_{50}) value of chromium for *Labeo rohita* as 3.5 ppm 96h [19]. Mercury exposure for long period caused severe morphohistological injuries to the olfactory system viz. degeneration of the receptor cells and non-sensory cells [20]. For both 96-hr LC_{50} and lethal concentrations, *Catla catla* were reported significantly more susceptible to Al toxicity, followed by that of *Labeo rohita* and *Cirrhinus mrigala* [21]. Metals may enter the fish through contaminated water and food intakes, start accumulating in liver, kidney, gills, skin, fins, muscles and bones [22]. In the present study, the impact of the pesticide could be observed by the behavioural changes like surfacing, erratic movement, increased mucous secretion, decreased opercular movement and loss of balance.

5. Conclusion

All the observations indicated the impact of mercury toxicity and caused behavioral alterations, those observed in this study these result in severe physiological problems, ultimately leading to the death of fish. Therefore, the present investigation demonstrates a relation among heavy metals stress, behavioral disorders, and survival and mortality rates. Hence this study can be used as a tool for creating awareness among the local farmers and compare the sensitivity of various species of aquatic animals and potency of effluent using LC_{50} values and to derive safe concentration so that the use of the highly toxic heavy metal can be minimized.

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7. References

1. Clarkson TW. Human health risks from methylmercury in fish. E T C. 1990; 9:821-823.
2. Martins RJE, Pardob R, Boaventura RAR. Cadmium (II) and zinc (II) adsorption by the aquatic moss *Fontinalis antipyretica*: effect of temperature, pH and water hardness. JWRP. 2004; 38:693-699.
3. Leland HV, Kuwabara JS. Trace metals. *In: Fundamentals of Aquatic Toxicology*, Hemsiphere, New York. 1985, 374-15.
4. Vahter MA, kesson A, Lind B, Bjo¨rs U, Schu¨tz A, Berglund M. Longitudinal study of methylmercury and inorganic mercury in blood and urine of pregnant and lactating women, as well as in umbilical cord blood. Environ Res Section A. 2000; 84:186-194.
5. Ghosh A, Sil PC. A protein from *Cajanus indicus* Spreng protects liver and kidney against mercuric chloride-induced oxidative stress. Biol Pharm Bull. 2008; 31:1651-1658.
6. Javed Md. Heavy Contamination of Freshwater fish and Bed Sediments in the River Ravi Stretch and Related Tributaries. Pak J of Biol Sci. 2005; 8(10):1337-1341.
7. Harmon SM, King JK, Gladden JB, Chandler GT, Newman LA. Mercury body burdens in *Gambusia holbrooki* and *Erimyzon sucetta* in a wetland mesocosm amended with sulfate. ELSEVIER. 2005; 59(2):227-233.
8. International Programme on Chemical Safety (IPCS). Environmental health criteria: methylmercury. Geneva (Switzerland) 7 World Health Organization. 1990, 101.
9. Reda FA, Bakr Ahmad M, Kamel Sayed A, Sheba, Doaa R. Abdul-Haleem., A mathematical model for estimating the LC50 or LD50 among an in seet life cycle Egypt. Acad. J Bio Sci. 2010; 32:75-81.
10. Atif F, Parvez S, Pandy S, Ali M, Kaur M, Rehman H *et al*. Modulatory effect of cadmium exposure on Deltamithrin-induced oxidative stress in *Channa punctatus* Bloch. Arch. Environ. Contam. Toxicol. 2005; 49:371-377.
11. Alkarkhi AF, Norli M, Ahmad I, Easa AM. Analysis of heavy metal concentrations in sediments of selected estuaries of Malaysia- a statistical assessment. Environ Monit Assess. 2009; 153:179-185.
12. Das KK, Banerjee SK. Cadmium toxicity in fishes, Hydrobiol. 1980; 75:117-121.
13. Biuki NA, Savari A, Mortazavi MS, Zolgharnein H. Acute toxicity of cadmium chloride on *Chanos chanos* and their behavior responses. World J Fis. Marine Sci. 2010; 2:481-486.
14. Pandi Prabha S1, Johanna Rajkumar. Hepato toxicity of lead in Indian major carp *Labeo rohita (Rohu)* - A Fourier transform infra-red study. ISSN: 0974-2115, JCPS. 2015; 8(2):317-323.
15. Kousar S, Javed M. Heavy metals toxicity and bioaccumulation patterns in the body organs of four fresh water fish species. Pak Vet J. 2014; 34(2):161-164.
16. Pathak Renu, Sadhu DN, Alam MN, Sadhu S. Toxicity of pesticides atus. Envt. And Ecol. 2007; 255(2):369-372.
17. Walia GK, Handa D, Kaur H, Kalotra R. Erythrocyte abnormalities in a Freshwater fish *Labeo rohita* exposed to tannery industry effluent. Intl. J of Pharm and Bio Sci. 2013; 3:283-295.
18. Nagaraju B, Sudhakar P, Anitha A, Haribabu G, Rathnamma VV. Toxicity evolution and behavioural studies of freshwater fish *Labeo rohita* exposed to Rinon. Intl. J of Research in Pharm and Bio Sci. 2011; 2(2):722-727.
19. Muthukumaravel K, Rajaram P. A study on the toxicity of chromium on the histology of gill and liver of freshwater fish *Labeo rohita* (Ham.). Intl J of Pure and Appli Zoo. 2013; 1(2):122-126.
20. Hansen JA, Rose JD, Jenkins RA, Gerow KG, Bergman HL. Chinook salmon (*Oncorhynchus tshawytscha*) and rainbow trout (*oncorhynchus mykiss*) exposed to copper; neuro physiological and histological effects on the olfactory system. Environ Toxicol Chem. 1999; 199(18):1979.
21. Azmat H, Javed M, Jabeen G. Acute toxicity of aluminium to the fish (*C. catla*, *L. rohita* and *C. mrigala*). Pak Vet J. 2012; 32:85-87.
22. Rauf A, Javed M, Ubaidullah M, Abdullah S. Heavy metal levels in three major carps (*Catla catla, Labeo rohita* and *Cirrhinus mrigala*) fromthe river Ravi, Pakistan. Pak Vet J. 2009; 29:24-26.