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Short communication

Determination of LC_{50} of cadmium chloride in *Heteropneustes fossilis*

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Abstract

The aim of present study was to determine the LC_{50} value of cadmium chloride in *Heteropneustes fossilis.* Experimental fishes were procured from fish markets/fish ponds, which were measured an average length 16±4 cm and weight 28±5 g. Thereafter they were kept in sterile aquaria and fed with fish meal @ of 4% body weight of fish. Seven groups of experimental fish (containing 10 fish in each group) were exposed to different concentrations of cadmium chloride i.e. 0, 30, 40, 50, 60, 70 and 80ppm respectively for 96 h. Simultaneously they were fed with dried fish meal. Physico-chemical parameters like D.O., Hardness, Chlorine etc. of aquaria water and the mortality rate of fish were monitored daily. It was found that the 50% lethal concentration (LC_{50}) of cadmium chloride for *Heteropneustes fossilis* is 50.41 ppm and LC_{100} (95% confidence limit) 86.32 ppm.

Keywords: LC_{50}; Cadmium chloride; Catfish; *Heteropneustes fossilis*, Toxicity

Introduction

The fish consumption has been increasing throughout the world with improved awareness among consumers and culture of fish; an important source of proteinaceous foods for human has become popular during the last few decades in entire Indian subcontinent (Kumar, 2007; Prasad and Kumar, 2007; Saxsena and Garg, 2010). In recent past there has been reports of poor quality fish with high metal content in their tissue, which can be important for the heath of the consumer. Toxic heavy metals are increasingly being released into the environment with the advent of agricultural and industrial revolution in India. A variety of contaminants including toxic heavy metals such as cadmium, copper, mercury and zinc are reported to be ubiquitously present in the waste water which are generally released into aquatic ecosystems and toxic for aquatic organisms (Olsson, 1998; Kumar *et al.*, 2007; Kumar *et al.*, 2009).

Among the toxic heavy metals present in water bodies, cadmium (Cd) is the most abundant and is an emerging global concern due to their potential hazards on the public health. It acts as a cumulative poison and is listed by the Environmental Protection Agency as one of 129 priority pollutants. Major sources of this toxicant include nickel-Cd and silver-Cd batteries manufacturing plants, sewage sludge and lead mining and processing units (Kumar *et al.*, 2009). In general, they are not biodegradable and therefore, their bioaccumulation in fish, oyster, mussels, sediments and other components of aquatic ecosystems have been reported from all over the world. It has been suggested that the problem of heavy metals accumulation in aquatic organisms including fish needs continuous monitoring (Seebaugh *et al.*, 2005).

Indian catfish *Heteropneustes fossilis* is a hardy, bottom dweller and omnivorous fish. It can thrive well even in shallow dirty muddy water bodies. People consume muscles and different fish organs. Very little information is available on LC_{50} of cadmium chloride in this fish species. The present study was aimed to determine the LC_{50} of cadmium chloride in *Heteropneustes fossilis*.

Materials and methods

This study was carried out in the Laboratory of Immunocytochemistry, Bareilly College, Bareilly. *Heteropneustes fossilis* was selected for this study by being bottom dwelling nature. *Heteropneustes fossilis* with average length of 25±4 cm and weight of 80±5g were procured from local fish market, Bareilly, India. The fishes were then bathed in 0.01% potassium permanganate ($KMnO_4$) solution for 15 minutes for two consecutive days to neutralize possible external infectious pathogenic microorganisms, followed by they were placed in plastic pools (500L) containing tap water and kept for fifteen days for acclimatization. The fish were fed at least once a day

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during this period. Physiochemical parameters like pH, temperature, dissolved oxygen and hardness of all aquaria water were observed.

Water quality parameters (temperature, dissolved oxygen (DO), salinity and pH) in the aquaria were also determined before and periodically during the tests. The water temperature was kept between 20°C and 22°C. Average water temperature was 21.7°C; average pH was 8.8; average salinity was 0.189% on day one and 0.290% on the last day; dissolved oxygen 8.3 mg/l and the experimental medium was aerated in order to keep the amount of oxygen not less than 4 mg/l. Water was renewed after every 24 hours after cleaning the aquaria and removing dead fish, if any.

LC_{50} Determination

The toxicant used in static bioassays was cadmium chloride ($CdCl_2 . H_2O$, Merck) in tap water. The test organisms (i.e. fish) were randomly distributed in aquaria (50L) filled with different concentrations of cadmium chloride solution ie 30, 40, 50, 60 and 80 ppm $CdCl_2$ and mortality was recorded at 24, 48, 72 and 96 h. Ten fishes were used per concentration and the experiment was conducted in triplicate. The aquaria were not aerated at the time of dosing with cadmium chloride. There was a simultaneous control group together with the actual experiments. The control group was kept in experimental water without adding the cadmium chloride, keeping all other conditions same. All experiments were carried out for a period of 96 h. The number of dead fish was counted every 12 h and removed immediately from the aquaria.

Percent mortality was calculated and the values were transformed into probit scale and analysed as per Finney, 1971. Regression lines of probit against logarithmic transformation of concentrations were obtained. Slope function (S) and confidential limits (upper and lower) of the regression line with Chi-square test (EPA, 1999) were calculated and also used SPSS 11 for LC_{50} value of cadmium chloride with the help of probit analysis.

Results

LC_{50} of Cadmium chloride ($CdCl_2$)

Table 1 shows the relation between the cadmium chloride concentration and the mortality rate of *Heteropneustes fossilis* according to Finney's Probit Analysis. Mortality in control group was virtually absent and found to be suitable for LC_{50} upper and lower confidence limits and fitted for regression equation along with slope function for 96 h exposed period.

Evaluation of the degree of scatter of observed LC_{50} values was 50.41, the lower and upper lethal confidence limits for cadmium chloride indicate a wide range of 41.45 to 58.56 ppm within which the concentration response for 96 h exposure (Table-2 and Fig-2). In the fitted regression equation 'b' values were directly related to the exposure period. The inclination of the regression lines towards horizontal position (slanting slopes) indicated that increase in the concentration of cadmium chloride enhanced mortality in *Heteropneustes fossilis*. Further, as there was an increase in regression coefficients, with reduction in lethal LC_{50} of Cadmium toxicity, Chi-square test showed that all values were well fit at 0.05 probability levels.

Conc.	Number of subjects	Observed Responses	Expected Responses	Residual	Probability
00.00	10	0.0	0.105	- 0.105	0.01046
30.00	10	2.0	1.748	0.252	0.17484
40.00	10	3.0	3.166	-0.166	0.31663
50.00	10	5.0	4.924	0.076	0.49239
60.00	10	7.0	6.697	0.303	0.66967
70.00	10	8.0	8.152	-0.152	0.81516
80.00	10	9.0	9.123	-0.123	0.91231

Table 1: Showing correlation between the Cadmium chloride ($CdCl_2$) concentration and the mortality rate of *Heteropneustes fossilis*

Estimated LC Values and Confidence Limits			
Point	Concentration(mg/l)	95% Confidence Limits	
		Lower	Upper
LC/EC 1.00	- 0.36948	- 43.15465	16.94507
LC/EC 5.00	14.50799	- 16.95248	27.71731
LC/EC 10.00	22.43912	- 3.14525	33.62100
LC/EC 15.00	27.79021	6.05051	37.72410
LC/EC 50.00	50.41625	41.44606	58.56014
LC/EC 85.00	73.04228	63.82415	92.41364
LC/EC 90.00	78.39338	68.02060	101.51604
LC/EC 95.00	86.32450	74.00565	115.24191
LC/EC 99.00	101.20198	84.84967	141.37230

Table 2: Showing LC_{50} value of Cadmium chloride ($CdCl_2$) with lower and upper (95%) confidence limits

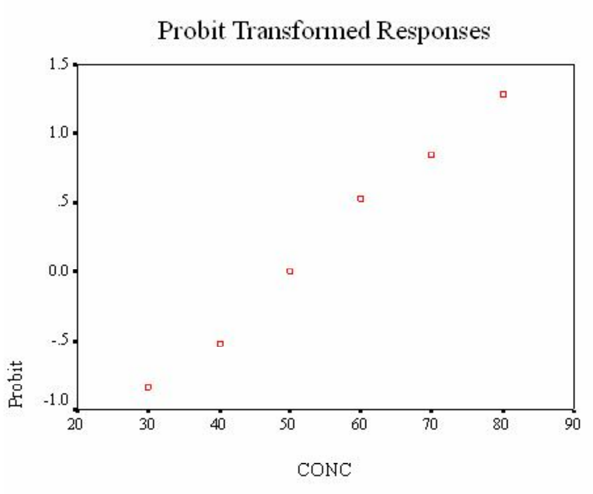

Fig. 1: Plot of adjusted probits and predicted regression line

Discussion

Susceptibility of catfish (*Heteropneustes fossilis)* to the lethal effect of cadmium chloride was duration and concentration dependent as mortality increased with an increase in its concentration. LC_{50} value of Cadmium chloride divulges the susceptibility of catfish to lethal concentration of cadmium, depicts that the toxicity is dilution and duration dependent. Higher percent of mortality occurred with increase in concentration and exposure period, hence confirms the observation made in case of salmonids, *Oncorhynchus mykiss, Salvelinus confluentus* and *Oncorhynchus tshawytscha* (Finalayson and Verrue, 1982; Hansen *et al*., 2002), guppy, *Poecilia reticulate* (Yilmaz *et al*., 2004), *Cyprinus cario* (Muley *et al*., 2000; Dardenne *et al*., 2007), Nile tilapia, *Oreochromis niloticus* (Garcia *et al*., 2006) and Rohu, *Labeo rohita* (Dutta and Kaviraj, 2001). It also concurs the Canadian Environmental Protect Act, 1994 report in which it has been suggested that toxicity of cadmium in fish varies from species to species.

We employed Finney's Probit Analysis for evaluating the acute toxicity response of cadmium chloride. Analysis gave 96 h LC_{50} value for *Heteropneustes fossilis* exposed to cadmium chloride concentrations as 50.41ppm. Control mortality was zero. Virtual absence of mortality in control group of *Heteropneustes fossilis* envisages that they are suitable for LC_{50} upper and lower confidence limits and suited for regression equation along with slope function for 96 h exposure duration. 95% lower and upper confidence limits for the LC_{50} were 74.00 ppm and 115.24 ppm respectively. The behavioral changes were also noted at high concentration of cadmium chloride. The toxic effects increased with the dose. Our results are in agreement with Yilmaz *et al*., 2004. Based on Finney's Probit Analyasis, they reported 30.4 mg/l value as 96 h LC_{50} value of cadmium on guppy (*Poecilia reticulate*). The other supporting studies are given by Oryan and Nejatkhah, 1997, Woodel *et al*., 1988; Muly *et al*., 2000.

The results of these studies may provide guidance to selection of acute toxicity to be considered in field biomonitoring efforts designed to detect the bioavailability of cadmium chloride and early warning indicators of Cadmium chloride toxicity in *Heteropneustes fossilis*.

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