Environment and Ecology 40 (4B) : 2397—2401, October—December 2022
ISSN 0970-0420

Acute Toxicity (96 hours) Study of Clofibric Acid on *Labeo rohita* (Hamilton 1822): Determination of LC_{50} Value and Behavioral Changes

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Received 1 August 2022, Accepted 7 September 2022, Published on 10 November 2022

ABSTRACT

Clofibric acid (CA) is one of the recalcitrant pharmaceutical compounds and was the first prescribed drug metabolite reported in the environment. By acute exposure (96 hours) to *Labeo rohita*, the LC_{50} value of CA is 710.157 mg/L. The fish displayed excitement and erratically moved about the aquarium within the first few hours after adding the CA. Opercular movement and mucus secretion both increased significantly. The rate of opercular movement in fish in the control tank was regular at 76 beats per minute, but it exceeded 114 beats per minute in the treatment tank. The fish moved jerkily and quickly when the CA concentration was high. All treatment tanks, except for the control tank, experienced frequent surfacing. Changes in body color were observed in high concentrations except for the control. The behavioral irregularities, displayed by the exposed fish increased with increasing concentrations of CA, thus, exhibiting a positive correlation with concentrations. The study observed no abnormal behavior in the control group of fish.

Keywords Clofibric acid, *Labeo rohita*, Acute exposure, LC_{50}, Behavioral irregularities.

INTRODUCTION

Pharmaceutical medications are typically created and distributed for use in human, veterinary, agricultural, and aquaculture procedures (Saravanan and Vijaya kumar 2012, Guerra and Quintela 2014). These medications are being dispersed into aquatic environments as parent chemicals, conjugates, or metabolites due to overproduction, reckless use, and disposal (Guerra and Quintela 2014). The pharmaceutical, chemical clofibric acid (CA) has recently been identified as an environmental pollutant due to its recalcitrance (Evangelista *et al*. 2010). CA is a metabolite of clofibrate and serves as a lipid regulator

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(Corcoran *et al*. 2015). Clofibric acid is excreted by people taking the drug Clofibrate (Heberer 2002) to prevent cardiovascular disease. Plasma lipoprotein and triglyceride concentrations in fish exposed to Clofibric acid may vary (Zimetbaum *et al*. 1991). For Swiss lakes and German rivers, the concentrations of CA in the surface water and from effluents of sewage treatment plants are 0.551 µg/land 1.6µg/L, ground (4µg/L), surface and drinking waters (0.07–0.27µg/L) all over the world. Although now an 'old' drug which has mainly been replaced, it is still one of the most frequently found and reported drugs in sewage effluents (up to the 1 µg/L level), ground (4 µg/L), surface and drinking waters (0.07–0.27 µg/L) all over the world (Tauxe-Wuersch *et al*. 2005). Clofibric acid is non-biodegradable, highly mobile and very persistent in the environment, with a half-life of 21 years and a water residence time of 1–2 years (Richardson and Bowron 1985, Buser *et al*. 1998, Winkler *et al*. 2001).

It has been suggested that future studies should concentrate on the toxicology of various medications (Brooks *et al*. 2009) and better understand the consequences of low pharmacological concentrations in aquatic species that are susceptible to them (Bain and Kumar 2014). A common and most important cultivated freshwater fish species, with a strong growth rate and high economic value in several nations, including Nepal, Myanmar, Pakistan, Bangladesh, and more so India, notably in the central and northern parts of the country, Rohu (*Labeo rohita*) is one of the Indian major carps (Sahoo *et al*. 2013). The occurrences and toxicity of numerous medicines and their derivatives in aquatic bodies are currently highly significant (Ramaswamy *et al*. 2011), and their toxicological effects on the physiology of aquatic creatures, particularly in freshwater fish, are relatively limited (Saravanan *et al*. 2011). Therefore, the current experiment is being carried out to evaluate the 96 hours LC_{50} of CA on Indian Major Carp *Labeo rohita* with cumulative mortality as well as behavioural responses.

MATERIALS AND METHODS

Experimental chemical

Clofibric acid (a-(p-Chlorophenoxy) isobutyric acid, CAS No. 882-09-7) is purchased from Hind Biotech company. The Dimethyl Sulphoxide is used as a solvent in the present experiment due to their low water solubility.

Procurement and acclimatization of experimental animal

The advanced healthy and uniform sized with an average weight of 28.6 ± 1.4 g (mean ± SD) and length 12.8 ± 1.2 cm (mean ± SD) *Labeo rohita* fingerlings were procured from Bengal Fish Hatchery of Sonarpur, Kolkata, was transported safely to wet laboratory (Dept of Aquatic Environment Management) of the Faculty of Fishery Sciences, through aluminium "Hundi" during the morning hours (6 am – 9 am) to avoid stress. For acclimatization, the fishes were gently transferred in two FRP tanks (1000 L capacity, 950 L water volume) after 2% disinfection with $KMnO_4$ treatment for three weeks. They were kept under constant temperature 16°C ± 0.5 and 12:12 h of photoperiod with continuous aeration. Fish were fed floating granule feed at a rate of 3% of body weight throughout this time, and the tanks were cleaned twice and had a 30% water exchange every alternate day to keep them free of disease and infection. An equal amount of water was also refilled to maintain the water's quality and level.

Experimental design

Acute toxicity studies

The acute toxicity (96 hours) was carried out in accordance with EPA guidelines (EPA 2002) in each glass aquarium (75 L capacity and 60 L volume) and simultaneously, the guidelines of animal ethics of West Bengal University of Animal and Fishery Sciences, Kolkata, India were sternly followed during the present study period and 7 fishes (each of 28.6 ± 1.4g and length 12.8 ± 1.2 cm) were introduced and feeding was withheld during the bioassay experiment. For, range finding test, the six concentration with duplicate were taken (680 mg/L, 690 mg/L, 700 mg/L, 710 mg/L, 720 mg/L, 730 mg/L) with a control (0 mg/L). After 24 hours, the test water was refilled, and a freshly made solution was added to keep the CA concentration constant. Every 24 hours, fish mortality and survival were noted. In the

experimental period (96 hours), the physico-chemical parameters (APHA 2005) were analyzed two times are as follows: The ranges of temperature, dissolved oxygen (DO), pH, free carbon dioxide, total alkalinity were 16–18°C, 5.7–6.0 mg /L, 7.3–7.6, 0.521–0.841 mg /L, and 159–178 mg /L, respectively during the short-term toxicity assay. After 6, 12, 24, 48, and 96 hours, observations were made, correspondingly. The cumulative mortality percentage of the experimental fish was recorded up to 96 hours of the experiment, and the behavioural changes were also noted. Scoop nets were used to remove dead fish. Finney's probit analysis was used to calculate the CA's 96 hours LC^{50} value on *Labeo rohita* (Finney 1971).

Statistical analysis

All values were analyzed by regression analysis and one-way analysis of variance (ANOVA) in MS excel to determine the significant differences ($P < 0.05$) among the concentrations on each parameter.

RESULTS AND DISCUSSION

Acute toxicity studies

An acute toxicity test of CA exposure was executed with the range finding test and carried out with six concentrations (680 mg/L, 690 mg/L, 700 mg/L, 710 mg/L, 720 mg/L, 730 mg/L) of CA with a control (0 mg/L). The mortality of fishes for CA doses was studied up to 96 hours and observations were recorded (6, 12, 24, 48, 72 and 96 hours) in fingerling. The range of LC_{50} was observed between 710 mg/L and 720 mg/L of CA. The cumulative mortality data for the LC_{50} test are presented in Table 1.

Table 1. Mortality of *L. rohita* in different concentration of CA (n=7 of each group of fishes).

Concentrations (mg/L)	No. of fish exposed	Log Concentration	Mortality (%) (96 h)	Probit
0	7	-	0	0
680	7	2.832	0	0
690	7	2.838	14	3.92
700	7	2.845	29	4.45
710	7	2.851	43	4.80
720	7	2.857	71	5.52
730	7	2.863	86	6.08

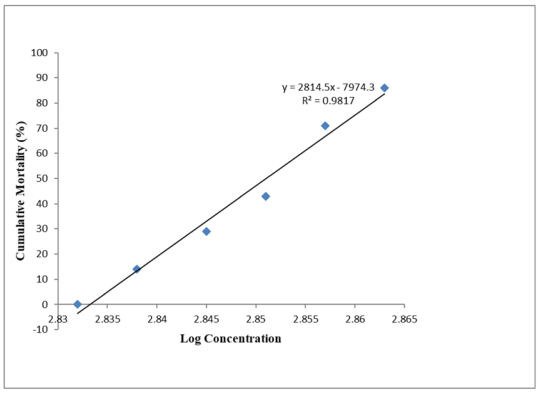

Fig. 1. 96 hours LC_{50} value of Clofibric Acid exposure on *Labeo rohita*.

The 96 hours LC_{50} value of Clofibric acid on *Labeo rohita* is 710.157 mg/L which is represented in Fig. 1. No. one has investigated the LC_{50} of CA on *Labeo rohita* in the world or in India, and only a small number of fish species have had their short-term toxicity investigated. According to (Nunes *et al*. 2005), the LC_{50} values for clofibric acid on *Gambusia holbrooki, Artemia parthenogenetica*, and *Tetraselmis chuii* are 526.5 mg/L, 87.22 mg/L, and 318.2 mg/L, respectively. *Gambusia holbrooki*, a mosquito fish, has a particularly durable character since it can endure high salinity levels. However, *Labeo rohita* is less hardy than *Gambusia holbrooki* and is not a euryhaline fish. By this investigation, it is very clear that Clofibric acid is not very acutely toxic to *Labeo rohita* and the maximum mortality occurs in very high concentration. Rohu fish can tolerate wide range of concentration of CA.

Behavioural study

When *Labeo rohita* is briefly exposed to Clofibric acid, behavioural changes are periodically seen and noted. It is proven that the exposed fish had distinct clinical toxicological symptoms, varied in severity based on the CA concentration. Following the addition of the chemical in the experimental tanks, the behavioural and morphological changes are noted. Every 30 minutes on the first day were recorded and observation continued up to 12 hours, then 24 hours, 48 hours and 96 hours. The observed behavioural changes are described in Table 2.

Table 2. Behavioural responses of *Labeo rohita* during CA exposure: (None −, low +, moderate + +, high + + +, very high + + + +).

Behaviour	Concentration (mg/L)						
	0	680	690	700	710	720	730
Rapid jerk Movement	-	+	+	+	+ + +	+ + + +	+ + + +
Jumping tendancy	-	+	+	+ +	+ + +	+ + + +	+ + + +
Opercular movement	-	+	+	+ +	+ + +	+ + +	+ + + +
Erratic swimming	-	+	+	+ +	+ + +	+ + + +	+ + + +
Mucus secretion	-	-	+	+ +	+ + +	+ + + +	+ + + +
Loss of equilibrium	-	-	-	+	+ +	+ + +	+ + + +
Frequent surfacing	-	+	-	+ +	+ +	+ + + +	+ + + +
Changes of body color	-	-	-	+	+ +	+ + +	+ + + +

During first few hours after adding the CA, the fish showed hyperactivity with erratic movements in aquarium. Mucus secretion and opercular movement was increased in very high level. In the fish of the control tank rate of the Opercular movement was normal 76 beats /min but in the treatment tank, the rate of Opercular movement of fish was more than 114 beats /min. Rapid jerk movement of the fish was observed in the high concentration of the CA. Frequent surfacing also occurred in the all treatment tanks except control tank. The behavioural irregularities, displayed by the exposed fish increased with increasing concentrations of CA thus, exhibiting a positive correlation with the concentration. No visible abnormal behaviour was observed in the control group of fish during the study. Several recent studies documented that pharmaceuticals and active neuro compounds have potential to change in the behaviours of aquatic species such as animal stay away from the center of the experimental tank and prefer to stay corner of the tank, shoal coordination, feeding nature, dark and light preference (Riehl *et al*. 2011). Respiratory distress, noticed in exposed fish, could be caused by mucous precipitation and neurological dysfunction of gill epithelia in response to the toxicant which resulted in high respiratory rate as reported by (Banerjee 2007). High Opercular ventilation has been reported by (Robinson 2009) concluded that the behavioural alterations of fish can provide important indices for ecosystem assessment and monitoring.

ACKNOWLEDGEMENT

The authors are really thankful to the Head of the Department of the Aquatic Environment Management, Faculty of Fishery Sciences, West Bengal University of Animal and Fishery Sciences for his extreme support and valuable encouragements.

CONCLUSION

CA is hazardous after a brief exposure but has a significant long-term harmful effect. The toxicity increases with both the chemical concentration and the length of exposure. These modifications might substantially impact *Labeo rohita's* capacity to function normally in their aquatic environment. Consideration should be given to the discharge of untreated sewage treatment water into natural water bodies. In contrast to the customary approach, effluent water containing prescription drugs should be treated using current, effective techniques. This effluent water can also change a water quality parameter, negatively impacting the diversity of phytoplankton and other aquatic animals.

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