DETERMINATION OF LC_{50} OF ETHOXYQUIN ON *OREOCHROMIS MOSSAMBICUS* (PETERS) FISH EXPERIMENTAL MODEL

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Abstract

Ethoxyquin (EQ) is a synthetic antioxidant that is included in some animal and human foods as a preservative to protect fats and fat-soluble vitamins from oxidative degradation. Many unfavourable side-effects have been observed in animals fed with EQ-containing feeds. Studies on the detrimental effects of EQ on vertebrates are alarmingly growing, but the effects of EQ in aquatic systems have rarely been described. In this study, LC_{50} 96 hr value of EQ for the freshwater fish *Oreochromis mossambicus* (Tilapia) was determined through probit analysis. The fish maintained in freshwater behaved as normal. When the fish was exposed to EQ, erratic swimming, abnormal posture, dis-balance, sluggishness, imbalance in posture, increase in surface activity, opercular movement, gradual loss of equilibrium and spreading of excess of mucus all over the surface of the body were observed. The results revealed that a lower concentration of EQ is found to be highly toxic to fishes.

Keywords

Ethoxyquin; *Oreochromis mossambicus*; Lethal Concentration; Probit Analysis

1.0 Introduction

The damage caused by any chemical substance in an organism is called toxicity. Toxicity tests are experiments designed to predict the concentrations of toxicant and its duration of exposure required to produce an effect (Cope *et al*., 2004). Toxicity is species specific, because individuals have different levels of response to the same dose of a toxic substance (Smith & Stratton, 1986). The toxicity bioassays are used to detect and to calculate the potential toxicological effects of chemicals on organisms. These tests provide a database that can be used to assess the risk associated with a situation in which the organisms live. A variety of methods have been developed to evaluate the hazard and potential toxicity of chemicals to organisms, such as acute toxicity test, sub-acute toxicity test or chronic toxicity test.

Acute toxicity is the severe effect suffered by an organism from short term exposure to toxic chemicals (Koprucu *et al.,* 2006). LC_{50} is the estimation of the dose/concentration necessary to kill 50% of a large population of the test species. Experimentally, this is done by administering a chemical at different doses to a group of organisms and then observing the resulting mortalities in a set time period like 24, 48, 72 and 96 hrs. The acute toxicity data are important and beneficial in the fixation of sub lethal concentrations for chronic toxicity tests (Akanksha Singh & Kannez Zahra, 2017).

Water pollution is a major global problem leading to worldwide causes of death and diseases. Water bodies can be polluted by a wide variety of substances, including pathogenic microorganisms, putrescible organic waste, plant nutrients, toxic chemicals, sediments, radioactive substances etc. The impact of toxic chemicals on water parameters may influence population density of aquatic ecosystems and affect the species diversity in the particular ecosystem. This pollution has negative effects on aquatic habitat and often impacts human-health and well-being. Toxins from the waste water can kill aquatic life or cause varying degrees of illness to those who consume these aquatic animals. Chemicals released to water bodies affect the aquatic system.

Ethoxyquin (EQ: 6-ethoxy-1, 2-dihydro-2, 2, 4-trimethylquinoline; E324) is an antioxidant in animal feeds that has been reported as a toxic substance if used above a particular concentration. EQ cannot be used in any food for human consumption (except spices, e.g., chilly), but it can pass from feed to farmed fish, poultry and eggs. Hence, human beings can be exposed to this antioxidant. Nevertheless, some harmful effects in animals and people occupationally exposed to it were observed in 1980's which resulted in the new studies undertaken to re-evaluate its toxicity. Initially registered as a pesticide in 1965, Ethoxyquin is a broadly used antioxidant and has been used as a post-harvest indoor application for fruits. Ethoxyquin is responsible for a wide range of health-related problems in dogs as well as in humans (Alina Blaszczyk *et al.,* 2013). The effect of Ethoxyquin in aquatic systems has rarely been described.

Fish as an aquatic vertebrate, is in direct contact with the aquatic environment and anything added to its environment may cause some impact on them (Gabriel *et al*., 2007). *Oreochromis mossambicus* (Peters) (Mozambique tilapia) is one of several tilapia species that are commonly cultured and are characterized by their easy adaptability to various environmental conditions (Kamal and Mair, 2005). The ease of propagation makes tilapia one of the most preferred cultured food fish worldwide.

Probit analysis is a type of regression used to analyze binomial response variables (Hahn & Soyer, 2008). Probit analysis is commonly used in toxicology to determine the relative toxicity of chemicals to living animals. This is done by testing the response of an organism under various concentrations of chemicals and then comparing the concentrations at which a response occurs. Probit method is widely accepted and the most accurate method for calculating LC_{50}. Therefore, the present study aimed to determine the LC_{50} of Ethoxyquin by probit analysis for the assessment of Ethoxyquin toxicity on freshwater fish *Oreochromis mossambicus* (Peters) experimental model for the behavioural changes observed in Ethoxyquin toxicity for a period of 96 hrs.

2.0 Methodology

2.1 Experimental fish

Oreochromis mossambicus (Peters) was collected from Kerala and maintained in the culture room. They were maintained in well-aerated tubs (40 litres capacity), which was dechlorinated and sustained with fresh water, maintaining light and dark at 12:12 hrs.

2.2. Determination of LC_{50} of Ethoxyquin for 96 hours

2.2.1. Collection and maintenance of fishes

Oreochromis mossambicus (Peters) was selected as an experimental animal. The fishes (5–10 gram weight) were collected from Kerala and maintained in the culture room. The fishes were checked against injury or infection by keeping in 0.2% of potassium permanganate solution for 2-4 min. The fishes were acclimatized in laboratory conditions for 6-10 days. During acclimatization the fishes were fed with a commercial diet. Fishes were not given feed 24h before the experiment.

2.2.2. Water quality parameter analysis

Preliminary screening and standardization of physicochemical features of tap water were carried out following the standardized procedures as prescribed by American Public Health Association guidelines (APHA, 1998), which were maintained throughout the treatment period.

2.2.3. Chemicals used

All reagents used were of analytical grade, purchased from local commercial sources and used without further purification. Ethoxyquin (1, 2-dihydro-2, 2, 4-tri methyl quinolin-6-yl ethyl ether) of 75% purity was purchased from Sigma Aldrich, Germany.

2.2.4. Evaluation of Medium Lethal Concentration of Ethoxyquin (LC_{50})

In order to find the median lethal concentration (semi-static; 96 h-LC_{50}) for 96 hrs duration, the acclimatized fish were transferred into six separate tanks. Fish were not fed 24 hr prior to the experiment in order to reduce the food and faeces contamination in the test solution. Ethoxyquin at seven different concentrations i.e., 5, 10, 15, 20 and 25 mg/L were exposed to fish maintained in separate tanks, 50 Litre capacity, holding 15 healthy fish per tank and maintained triplicates under the same conditions. Along with the treatment groups, one control group, namely toxicant free group, was also maintained. The mortality and behavioural changes of fish from the experimental and control groups were monitored regularly at every 24 hr interval up to 96 hr duration. The concentration at which 50% mortality of fish represents the median lethal concentration (96 hr-LC_{50}), which was further confirmed using probit tool of regression analysis with a confident limit of 5% level (Finney, 1971).

3.0 Results and discussion

3.1. Water Quality Analysis

The tap water was taken for inducing ethoxyquin toxicity. The different water quality parameters were analysed prior to the induction of ethoxyquin toxicity in order to check the quality of water used for the LC_{50} assessment. The results of the water analysis have been summarized in table 1.

Table 1: Results of Water Quality Analysis.

S. No	PARAMETERS	UNIT	OBSERVED VALUE	STANDARD LIMIT
1	Colour	Colour units Hz	1	5 Hz Units
2	Odour	---	Agreeable	Agreeable
3	pH	---	7.4	6.5 – 8.5
4	Electrical conductivity	µS/cm	273	0 - 800
5	Total hardness	mg/L	165	300.0
6	Calcium hardness	mg/L	26.0	75.0
7	Magnesium hardness	mg/L	24.3	30.0
8	Chlorides	mg/L	10.6	250.0

9	Total dissolved solids	mg/L	175	500.0
10	Sulphates	mg/L	16	200.0
11	Alkalinity	mg/L	90	200.0

All the results of water quality parameters performed in the experiment falls within the standard limit. This reveals that the water can be used for growing the fishes and also for inducing ethoxyquin toxicity. An appropriate assessment of the water quality parameters, especially chlorides, is very important prior to the LC_{50} determination. Presence of chloride beyond the standard limit is detrimental to fishes. It was found that chloride level falls within the normal limit suggesting the water is suitable for present study.

3.2. Probit Analysis for LC_{50} Assessment

Fishes were exposed to different concentrations of ethoxyquin for 96 hrs and the percentage of fish mortality was summarized in table 2.

Table 2: Percentage of mortality exposed at different concentrations of EQ in *Oreochromis mossambicus* for 96 h.

S. No	Concentration (mg/L)	Total number of fish	Mortality (%)	Time for Mortality (hours)
1	5	15	20	96
2	10	15	31.11	96
3	15	15	62.22	96
4	20	15	77.78	96
5	25	15	91.11	96

The mean mortality and percentage mortality with standard deviation for *Oreochromis mossambicus* against ethoxyquin was calculated using statistical tools and the results have been shown in table 3.

Table 3: Mean Mortality and Percentage Mortality for *Oreochromis mossambicus* against EQ

Observed Parameters	Ethoxyquin Concentration (mg/L)				
	5	10	15	20	25
Mean Mortality with SD	3.00±1.08	4.67±0.58	9.33±0.58	11.67±0.58	13.67±0.58
Percentage Mortality with SD	20±1.08	31.11±0.58	62.22±0.58	77.78±0.58	91.11±0.58

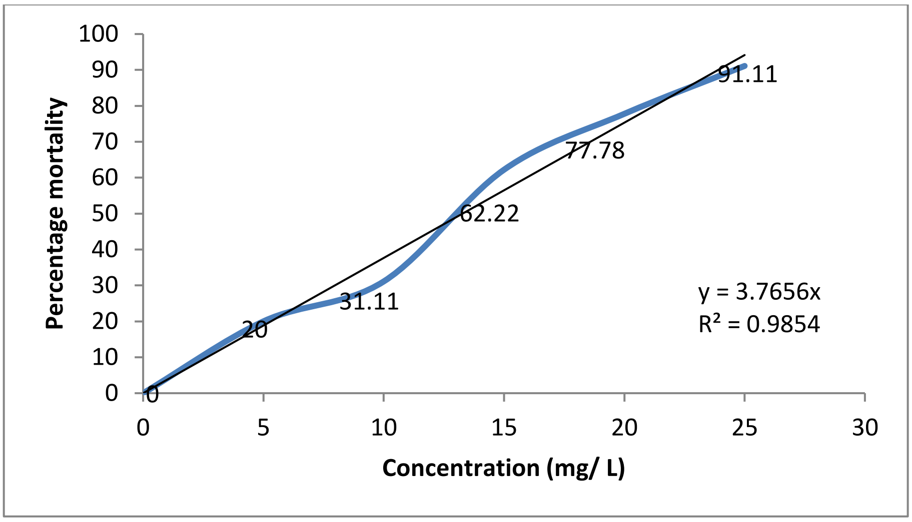
Toxicity of Ethoxyquin against *Oreochromis mossambicus* was determined by assessing the LCL (Lower Concentration Limit), UCL (Upper Concentration Limit) and Chi-Square test. The P-Value (table 4) was less than 0.05, which falls in the significant level. The whole procedure was done using SPSS 16.0 software.

Test substance	LC_{25} (LCL-UCL)	LC_{50} (LCL-UCL)	LC_{90} (LCL-UCL)	CHI SQUARE VALUE	P VALUE	R^2 VALUE
Ethoxyquin	6.880 (2.679-9.684)	11.377 (7.320-15.640)	29.586 (20.704-105.803)	12.297	0.006	0.9854

Table 4: Toxicity of Ethoxyquin against *Oreochromis mossambicus*

Median lethal concentration (LC_{50}-96 hrs) of ethoxyquin in *Oreochromis mossambicus* (N=15 fish in group) was calculated by plotting the concentration of Ethoxyquin Vs Percentage Mortality (Figure 1).

Fig 1: Estimation of LC_{50} at different exposure period



The LC_{50} was found to be 11.37mg/L. Lethal Concentration 50 (LC_{50}) is a standard toxicity dose measurement. This is the concentration of a chemical that kills 50% of a test population within a set period of time, usually 24 to 96 hours. Not all chemical poisoning results in the immediate death of an animal.

Some behavioural changes were also observed during the experiment. After 24 hr of ethoxyquin treatment, slow movement of the fish was observed along with behavioural abnormalities such as frequent engulfing of air, mucous secretion throughout the body, lethargic and bulging of eyes. During the entire study, abnormal behavioural patterns were observed. Immediately after the exposure to ethoxyquin, fishes showed immediate slow movement in swimming and remained in static position for a while. After some time, fishes showed erratic swimming and jumping to avoid the toxic environment. As they failed, then the fishes moved on the surface with a wide opening of gill operculum to engulf air.

4.0 Conclusion

The present study focuses on the determination of LC_{50} value of ethoxyquin on *Oreochromis mossambicus* (Peters) by probit Analysis. Probit Analysis is a type of regression used with binomial response variables. It is very similar to log it, but is preferred when data are normally distributed. Most common outcome of a dose-response experiment in which probit analysis is used is the LC_{50}/LD_{50}. Probit analysis can be done by eye, through hand calculations or by using a statistical program. The present study was an attempt to find the toxicity of Ethoxyquin on *Oreochromis mossambicus* (Peters) and the results conclusively showed that the Ethoxyquin is highly toxic to fishes even at very low concentration.

The fishes showed mortality at low concentration and with decrease of duration of exposure the fishes exhibit mortality at higher concentration. It has been reported earlier that pesticides, chemicals and xenobiotic accumulated in natural waters, which results in toxicity to aquatic organisms. The LC_{50} can be used as a relative measure to study the impact of the heavy metal concentration on test fishes at different intervals. This toxicity test on the effect of ethoxyquin on *O. mossambicus* offers a rapid method for assessing the heavy metal impact on this fish.

In the present study, the toxic effect of Ethoxyquin on *Oreochromis mossambicus* (Peters) was determined by the assessment of LC_{50} values calculated at different exposure periods. This type of preliminary investigations can be useful for deriving the safe level of various chemicals that can be released into the aquatic environments. The change in behavioural pattern of *Oreochromis mossambicus* (Peters) suggests that fish tried to defend against exposure to the ethoxyquin toxicant. This study on fishes will be very useful to provide a future understanding of ecological impact. Similar studies can be done to estimate the dose of human exposure and level of chemical residue that can be allowed in aquatic environments.

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