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Assessment of Lc50 of Ethoxyquin on Experimental Model, *Oreochromis mossambicus* and Impact of Sublethal Concentration of Ethoxyquin on Water Quality Parameters

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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 growing, but the effects of EQ in aquatic systems have rarely been described. In the present study LC_{50} 96 hr value of EQ to the freshwater fish *Oreochromis mossambicus* (Thilapia) was determined through Probit analysis. The fish maintained in freshwater behaved normal as usual. But 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 water quality parameters were also changed drastically after EQ toxicity induction. 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; Sub lethal concentration; Water Quality Parameters

1. 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 a*l.,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 data base 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 administrating a chemical at different doses to a group of organisms and then observing the resulting mortalities in a set time periods like 24, 48 72 and 96 h. 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 cause 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 ecosystem 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 off 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-2,2,4trimethyl qui -nolinq; E324) is a antioxidant in animal feeds 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., chili), but it can pass from feed to farmed fish, poultry, and eggs, so 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 reevaluate 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* (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. Therefore the present study aimed to determine the LC50 of Ethoxyquin by probit analysis for the assessment of Ethoxyquin toxicity on experimental model, *Oreochromis mossambicus* and to study the behavioural changes in *Oreochromis mossambicus* and water quality parameters on Ethoxyquin toxicity induction for a period of 96hr.

2. METHODOLOGY

2.1Experimental fish

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

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

2.2.1. Collection and maintenance of fishes

Oreochromis mossambicus was selected as an experimental animal. The fishes (5 –10 gram weights) 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 commercial diet. Fishes were not given feed 24h before experiment.

2.2.2. Water quality parameters 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. Chemical 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-trimethylquinolin- 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 h duration, the acclimatized fish were transferred into six separate tanks. Fish were not fed 24 h 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 L 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, were also maintained. The mortality and behavioural changes of fish from the experimental and control groups were monitored regularly at every 24h interval up to 96 h duration. The concentration at which 50% mortality of fish represent the median lethal concentration (96 h-LC50), which was further confirmed using Probit tool of regression analysis with a confident limit of 5% level (Finney, 1971).

2.2.5. Evaluation f Water Quality Parameters After Induction of Sublethal Concentration of Ethoxyquin

Based on the median lethal concentration for 96 hr duration, sublethal concentrations representing one-tenth of 96 h-LC_{50} was chosen as test concentration to evaluate water quality parameters. Fishes were divided into five groups, with nine specimens in each group. Group I was negative control group (without toxicant). In group II to VI sub lethal concentration of 96h – LC_{50} (ie, 1/10th of medial lethal concentration) was added and exposed to various time intervals like 24hr, 48hr,72hr and 96hr . The Important Water Quality Parameters Both Physical and Chemical were determined after the time of exposure and was compared.

3. RESULTS AND DISCUSSION

3.1. Water quality analysis

The tap water was taken for inducing ethoxyquin toxicity. The different water quality parameters were analyzed prior to the induction of ethoxyquin toxicity in order the check the quality of water used for the LC50 assessment. The results of the analysis were summarized in the table 1.

Table 1: Results of water quality analysis

SL. 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 LC50 determination. Presence of chloride beyond 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.

3.1.2. Probit Analysis for LC50 assessment

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

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

Sl. 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 were as shown in table 3.

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

Observed parameters	Ethoxyquin concentration in 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

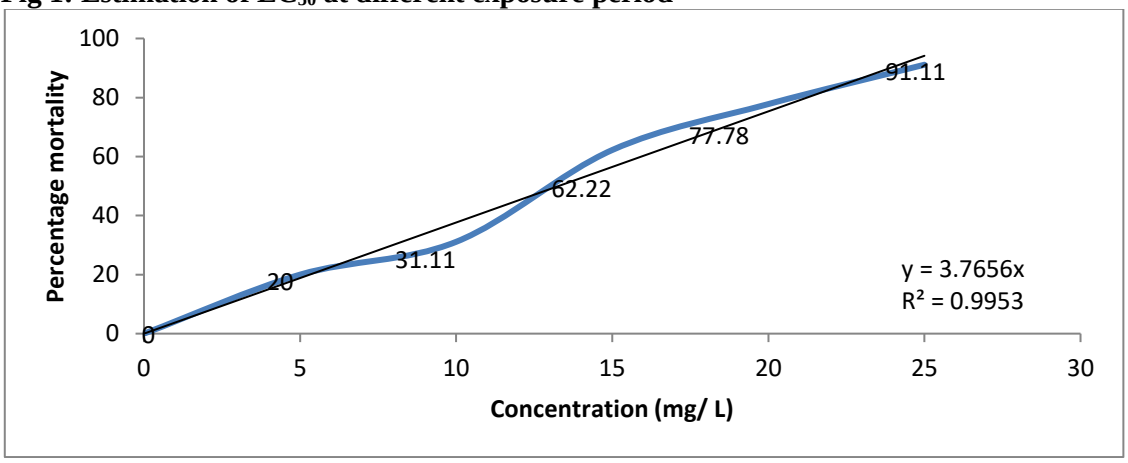
Toxicity of Ethoxyquin against *Oreochromis mossambicus was determined by assessing the LCL (*Lower Concentration Limit) , UCL (Upper Concentration Limit) and Chi-Square test. The P-Value (table 4) was less than 0.05 which falls in the significant level. The whole procedures was done using software SPSS.

Table 4: Toxicity of Ethoxyquin against *Oreochromis mossambicus*

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

Median lethal concentration (LC_{50}-96 hrs) of ethoxyquin in *Oreochromis mossambicus* (N=15 fish in group) was calculated by plotting 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 result in the immediate death of an animal.

3.1.3. Behavioural changes in *Oreochromis mossambicus* on EQ toxicity induction

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 as frequent engulping of air, mucous secretion throughout the body, lethargic and bulging of eyes. During the entire study abnormal behavioural pattern was 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 from the toxic environment. As they failed, then the fishes moved on the surface with wide opening of gill operculum to engulp air.

3.1.4. Evaluation of water quality parameters after EQ toxicity induction

The water quality parameters were analyzed after toxicity induction at every time of exposure. The results of water quality parameters after toxicity induction for each exposure time were summarized in table 5.

Table 5: Results of water quality analysis after toxicity induction

Sl No	Parameters	Time of Exposure				
		control	24hr	48hr	72hr	96hr
1	colour	1 Hz	1 Hz	1 Hz	1 Hz	1 Hz
2	odour	Agree able	Agreeable	Agree able	Agree able	Agree able
3	Turbidity max (NTU)	0.1	0.1	0.1	1.2	2.2
4	Electrical conductivity (µS/cm)	283	352	398	412	460
5	pH	7.2	7.3	7.4	7.7	7.9
6	total hardness (mg/l)	115	120	125	127	130
7	Ca hardness (mg/l)	26	22	21	22	22
8	Mg hardness (mg/l)	14.8	15.8	16.5	15.9	18.2
9	iron max (mg/l)	0.04	0.08	0.13	0.19	0.39
10	Cl max (mg/l)	10.6	11.2	11.3	11.6	11.6
11	TSS(mg/l)	50	40	43	40	49
12	TDS(mg/l)	175	192	198	199	205
13	Sulphates (mg/l)	16	20.7	25	21	20.7
14	Alkalinity (mg/l)	90	89	98	125	145

It was found that there was a drastic change in the water quality parameters on the induction of ethoxyquin for different time period. There was a significant change in pH and alkalinity was noticed. . The water quality affected the production traits of fish in aquacultures (Davies and Ansa, 2010). In our experiment it was observed that the pH and alkalinity of ethoxyquin treated water were increased when compared with control. During the experimental period, the fishes discharged their metabolic waste products as well as the unconsumed food materials in the surrounding water, which may have caused the increase of pH and alkalinity.

4. CONCLUSION

The present study focuses on the determination of LC_{50} value of ethoxyquin on *Oreochromismossambicus* by Probit Analysis.Probit Analysis is a type of regression used with binomial response variables. It is very similar to logit, 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* and the results conclusively showed that the Ethoxyquin is highly toxic to fishes even at very low concentration. The study on fishes will be very useful to provide a future understanding of ecological impact. In the present study the toxic effect of Ethoxyquin on *Oreochromis mossambicus* was determined by the assessment of LC_{50} values calculated at different exposure period. 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 pesticide,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. 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* suggests that fish tried to defences against exposure to the toxicant, ethoxyquin. The present study also tried to analyze the changes in physico-chemical parameters of water on exposure of sublethal concentration of EQ. The physic-chemical nature of water changed due to not only the addition of EQ, but also the discharge of metabolic wastes, faecal matters of fishes and unconsumed food materials in the water. The study on fishes will be very useful to provide a future understanding of ecological impact. Similar studies can be used to estimate the dose of human exposure and level of chemical residue that are allowed in aquatic environment.

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