Available online at www.ijpab.com

DOI: http://dx.doi.org/10.18782/2582-2845.8019

ISSN: 2582 – 2845

Ind. J. Pure App. Biosci. (2020) *8*(2), 33-37

Research Article

Indian Journal of Pure & Applied Biosciences

Assessment of Toxic Nature and Safe dose of Oxytetracycline and Garlic to an Indian Air-breathing Catfish, *Clarias batrachus* (Linnaeus)

Dina Nath Pandit* and Kumari Priya

Department of Zoology, VKS University, Arrah, Bhojpur, Bihar – 802 301, India

*Corresponding Author E-mail: panditdina@gmail.com

Received: 1.03.2020 | Revised: 3.04.2020 | Accepted: 8.04.2020

ABSTRACT

An initial work was conducted to assess toxic nature of oxytetracycline and garlic to an Indian air-breathing fish, Clarias batrachus (Linnaeus) (55.0±5.0g and 20.0±1.5cm). The physico-chemical parameters of experimental water were found within the limits. 96hr-LC_{50} dose of fish was determined by Behrens-Karber and Finney's method. The ideal median 96hr-LC_{50} value was determined 663.8 mg L^{-1} and 7391.36mg kg^{-1} for oxytetracycline and garlic respectively. Oxytetracycline was more toxic to the fish in relation to time and dose than garlic. The results indicated that oxytetracycline is moderately/slightly toxic but garlic seems least toxic/practically non-toxic for this fish. Large variations in the safe levels for the test materials were recorded. Therefore, precautions should be taken when high concentrations of oxytetracycline and garlic are used in chronic treatment of Clarias batrachus.

Keywords: *Clarias batrachus, Oxytetracycline, Garlic, Acute toxicity, Safe dose.*

INTRODUCTION

Intensive culture has been introduced to support worldwide demand of fish. A large number of feed additives are available to reinforce fish growth. Many antibiotics are used in this context. The utilization of antibiotics seems essential to check or treat fish diseases and to promote fish growth and health (Romero et al., 2012). Chloramphenicol, Oxytetracycline and Erythromycin is used by most of the fish farmers. Chloramphenicol is used to treat bacterial infections in fish and other purposes in pisciculture (Kreutzmann 1977; Barros-Becker et al., 2012). However, critical issues are raised on the application of antibiotics due to its side effects on aquatic ecosystems. So, attention has being shifted to phytobiotics as a viable alternative to antibiotics (Ramadu & Dash 2013). Many workers have reported the beneficial effects of garlic as dietary additives in fish (Jahanjoo et al., 2018).

But, report on toxic nature of oxytetracycline and garlic is lacking in fishes. Therefore, *Clarias batrachus* (Linnaeus), one of the most common Indian air-breathing catfish was selected to study its acute toxicity and safe level.

This study will help to establish the optimum dose of antibiotics/phytobiotics to ensure the production of healthy fish and safety for man.

Cite this article: Pandit, D.N., & Priya, K. (2020). Assessment of Toxic Nature and Safe dose of Oxytetracycline and Garlic to an Indian Air-breathing Catfish, *Clarias batrachus* (Linnaeus), *Ind. J. Pure App. Biosci. 8*(2), 33-37. doi: http://dx.doi.org/10.18782/2582-2845.8019

MATERIALS AND METHODS

Acclimation of animals: Live specimens of *Clarias batrachus* (Linnaeus) (55.0±5.0g and 20.0±1.5cm) were purchased from the local market of Arrah (Bhojpur), Bihar during November 2017 to January 2019. They were acclimatized for a fortnight in Departmental Laboratory of VKS University, Arrah. The fishes were disinfected properly and transferred to large aquaria and fed with fish food. Ethical guidelines were followed during the work.

Experimental Design: Oxytetracycline (500mg capsule) (Wockhardt Health Care Limited, Chennai, India) was purchased, decapsulated and stored. Fresh bulbs of garlic (*Allium sativum*) were purchased from an open market. The bulbs were dried under shade for one week. The dried bulbs were crushed, homogenized, sieved and stored.

The physico-chemical parameters of water used during the experimental works were determined using standard methods (A.P.H.A. 2009). LC_{50} dose was determined by two standard methods (Behrens 1929 & Karber 1931; Finney 1971). The safe level estimation after 96hr exposure of test materials was determined following standard methods (C.C.R.E.M. 1991; N.A.S./N.A.E. 1973).

Statistical analysis was done with Graph Pad Prism 5 software.

RESULTS AND DISCUSSION

Physico-chemical characteristics of water: The physicochemical characteristics of test water having temperature of 25.0±1.4^{0}C, pH: 7.67±0.19, dissolved oxygen: 6.61±0.21 mg L^{-1}, total alkalinity: 73.0±4.2mg L^{-1}, hardness: 230.67±55.26mg L^{-1} and chloride: 16.7±0.2mg L^{-1} was recorded during experimental period. The observed parameters are within in range for favourable growth performances have been documented (Boyd 1981). Physiological parameters like concentration and formulation of antibiotics and phytobiotics and its exposure also substantially effect living organisms (Gupta et al., 1981).

96hr-LC_{50} dose and toxic nature: LC_{50} values recorded in this study were attributed to size of fishes with potentially immune system for biotransformation of test substances from the body.

(I) Behren-Karber method: It is a non-parametric method. Equal spacing of the interval of dose and the equal number of fish at each dose was applied for observation from 0 to 100 %.

(A) 96hr-LC_{50} dose of oxytetracycline = 680.0 mg L^{-1} (Table 1).

(B) 96hr-LC_{50} dose of garlic = 413.75mg $fish^{-1}$ (7522.73mg/kg) (Table 2).

(II) Finney method: It is a parametric method in which after calculating percent mortalities, net/corrected percent mortalities from 10% to 100% were calculated. Then, values of empirical probit from 3.72 to 8.72 were noted from Fischer and Yates's table. From the values of empirical probit expected/provisional probit from 3.42 to 6.68 was recorded. Then, the values of working probit (from 3.82 to 6.64) and weighing coefficient (from 0.204 to 0.614) were calculated. Coincidently, these values were found similar in case of both oxytetracycline and garlic.

(A) 96hr-LC_{50} dose of oxytetracycline: A slope was calculated to be 6.53. Finally the median lethal concentration was calculated to be LC_{50} = Antilog 2.813= 647.6 mg/L of Oxytetracycline (range: 226.67 to 1850.19 mg/L) (Table 4).

(B) 96hr-LC_{50} dose of garlic: A slope was calculated to be 8.0. Finally the median lethal concentration was calculated to be LC_{50} = Antilog 2.6013= 399.3 mg/fish or 7260mg/kg of garlic (range: 266.33 to 598.65 mg/fish or 4842.36 to 10884.55mg/kg) (Table 5).

The observations showed that oxytetracycline was more toxic to the fish in relation to time and dose than garlic. The results indicated that oxytetracycline is moderately/slightly toxic but garlic seems least toxic/practically non-toxic for *Clarias batrachus*.

96hr-LC_{50} dose of Oxytetracycline ranged from 62.5-100.0mg/l in different fishes (Brain et al., 2004; Ankley et al., 2007). According to Carraschi et al., (2011) oxytetracycline cause environmental intoxication risk considering the lowest (50.0 mg/kg) and the highest predicted environmental concentration (1750 mg/kg)

because of its the >1 quotient. On the other hand, 96hr-LC_{50} of garlic for *Cyprinus carpio* was estimated to be 253.19 mg/L by Furthermore, Fridman et al., (2014) reported that bathing of *Gyrodactylus turnbulli* infected *Poecillia reticulata* in 7.5 and 12.5 ml/L garlic extract significantly reduced the infection in the fish. Garlic oil feeding of 100mg/kg in rats after 24 hr was found lethal by Joseph et al., (1989). From this account, it may be inferred that the differences in LC_{50} values in the sensitivity of different fish species to the type of toxicants exposures. It seems to be influenced by the species and age of the fish chosen for the toxicity tests.

Safe level of oxytetracycline and garlic: It is reported that, safe levels are added to account for uncertainties in data and evaluation processes. A range of safe level of oxytetracycline was calculated from 6.638 x 10^{-3} to 33.19 mg L^{-1} in *Clarias batrachus*. Similarly, a range of safe level of garlic was calculated from 73.914 x 10^{-3} to 369.568 mg kg^{-1} in *Clarias batrachus* (Table 3). The range indicates that it is difficult to decide the acceptable concentration of either oxytetracycline or garlic in *Clarias batrachus* (https://en.wikipedia.org/wiki/Toxicity).

Table 1: Behren-Karber method for 96hr-LC_{50} determination of Oxytetracycline for *Clarias batrachus* (body weight: 55.0g)

Group	Dose of Oxytetracycline (mg/l)	Difference between two consecutive dose (A)	No. of fish exposed	Mortality				Overall mortality at 96hr	Mean mortality between two consecutive dose (B)	A x B
				24hr	48hr	72hr	96hr			
1	0	0	10	0	0	0	0	0	0	0
2	400	200	10	0	0	0	1	1	0.5	100.0
3	600	200	10	0	1	2	4	4	2.5	500.0
4	800	200	10	1	4	6	7	7	5.5	1100.0
5	1000	200	10	3	6	8	9	9	8.0	1600.0
6	1200	200	10	6	8	9	10	10	9.5	1900.0
										5200.0

96hrLC50 = LC100 - $\frac{\Sigma AB}{N}$ = 1200 – $\frac{5200}{10}$ = 1200 – 520 = 680 mg/L oxytetracycline.

Table 2: Behren-Karber method for 96hr-LC_{50} determination of garlic for *Clarias batrachus* (body weight: 55.0g)

Group	Dose of garlic (mg/l)	Difference between two consecutive dose (A)	No. of fish exposed	Mortality				Overall mortality at 96hr	Mean mortality between two consecutive dose (B)	A x B
				24hr	48hr	72hr	96hr			
1	0	0	10	0	0	0	0	0	0	0
2	350	175	10	0	0	0	1	1	0.5	87.5
3	400	50	10	0	1	2	4	4	2.5	125.0
4	450	50	10	1	4	6	7	7	5.5	275.0
5	500	50	10	3	6	8	9	9	8.0	400.0
6	550	50	10	6	8	9	10	10	9.5	475.0
										1362.5

96hrLC50 = LC100 - $\frac{\Sigma AB}{N}$ = 550 – $\frac{1362.5}{10}$ = 550 – 136.25 = 413.75 mg/fish (7522.73mg/kg) garlic.

Table 3: Estimation of safe levels of garlic and oxytetracycline at 48/96hr exposure of *Clarias batrachus* (body weight: 55.0g)

S.	Method	Average Dose of		Accumulation Factor		Range of Safe level	
		Oxytetracycline (mg/L)	Garlic (mg/kg)	Oxytetracycline (mg/L)	Garlic (mg/kg)	Oxytetracycline (mg/L)	Garlic (mg/kg)
1.	CCREM (1991)	96hr-LC50 = 663.8	96hr-LC50 = 7391.36	0.05	0.05	663.8x 0.05 = 33.19	7391.36x 0.05 = 369.568
2.	(NAS/NAE) (1973)	96hr-LC50 = 663.8	96hr-LC50 = 7391.36	0.00001	0.00001	663.8x 0.00001 = $6.638x10^{-3}$	7391.36x 0.00001 = $73.914x10^{-3}$

Table 4: Probit analysis for toxicity of Oxytetracycline in *Clarias batrachus*

Dose of Oxytetra-cycline (mg/L)	Log dose of Oxy-tetracycline (mg/L)	Number of fish exposed	Mortality of fish	% of mortality of fish	Net/corrected mortality of fish	Empirical probit	Expected/Provisional probit	Working probit	Weighing Coefficient	nw	nwx	nwy	nwx^2	nwy^2	nwxy
	x	n		p			Y	y	w						
0	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0
400	2.602	10	1	10	10	3.72	3.42	3.82	0.238	2.38	6.19	9.10	16.11	34.73	23.68
600	2.778	10	4	40	40	4.75	4.75	4.72	0.614	6.14	17.06	28.98	47.38	136.79	80.51
800	2.903	10	7	70	70	5.52	5.54	5.48	0.564	5.64	16.37	30.91	47.53	169.37	89.73
1000	3.000	10	9	90	90	6.28	6.32	6.34	0.334	3.34	10.02	21.18	30.06	134.25	63.54
1200	3.079	10	10	100	100	8.72	6.68	6.64	0.204	2.04	6.28	13.55	19.34	89.94	41.72
	-	-	-	-	-	-	-	-	-	19.54	55.92	103.72	160.39	565.08	299.18

96hr-LC_{50} = Antilog 2.813= 647.6mg/L of Oxytetracycline.

Table 5: Probit analysis for toxicity of garlic in *Clarias batrachus*

Dose of Garlic (mg/L)	Log dose of garlic (mg/L)	Number of fish exposed	Mortality of fish	% of mortality of fish	Net/corrected mortality of fish	Empirical probit	Expected/Provisional probit	Working probit	Weighing Coefficient	nw	nwx	nwy	nwx^2	nwy^2	nwxy
	x	n		p			Y	y	w						
0	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0
400	2.602	10	1	10	10	3.72	3.42	3.82	0.238	2.38	6.05	9.10	3.53	34.73	23.13
600	2.778	10	4	40	40	4.75	4.75	4.72	0.614	6.14	15.98	28.98	10.51	136.79	75.41
800	2.903	10	7	70	70	5.52	5.54	5.48	0.564	5.64	14.96	30.91	13.68	169.37	82.00
1000	3.000	10	9	90	90	6.28	6.32	6.34	0.334	3.34	9.01	21.18	14.32	134.25	57.15
1200	3.079	10	10	100	100	8.72	6.68	6.64	0.204	2.04	5.59	13.55	14.52	89.94	37.11
	-	-	-	-	-	-	-	-	-	19.54	51.59	103.72	136.32	565.08	274.80

96hr-LC_{50} = Antilog 2.6013= 399.3mg/fish (7260.0mg/kg) garlic.

CONCLUSION

The acute toxicity studies are first step to determine the water quality requirements of fish that cause fish mortality. The present study showed that oxytetracycline is more toxic to *Clarias batrachus* than garlic. Therefore, precautions should be taken when high concentrations of oxytetracycline and even garlic are used in chronic treatment of fish.

REFERENCES

A.P.H.A. (2009). *Standard Methods for the examination of water and wastewater*. American Public Health Association, Washington, D.C.

Ankley, G.T., Brooks, B.W., Huggett, D.B., & Sumpter, J.P. (2007). Repeating history: pharmaceuticals in the environment. *Environmental Science & Technology 41*, 8211–8217.

Barros-Becker, F., Romero, J., Pulgar, A., & Feijo´o, C.G. (2012). Persistent oxytetracycline exposure induces an inflammatory process that improves regenerative capacity in Zebra fish larvae. *PLoS ONE.* 7:e36827. doi: 10.1371/journal.pone.0036827.

Behrens, B. (1929). Zur Auswertung der Digitalisblätter im Froschversuch. Naunyn-Schmiedebergs Archiv für Experimentelle Pathologie und Pharmakologie *140*, 237-256.

Boyd, C.E. (1981). *Water quality in warm water fish ponds*. Auburn University, Alabama, USA.

Brain, R.A., Johnson, D.J., Richards, S.M., Sanderson, H., Sibley, P.K. & Solomon, K.R. (2004). Effects of 25 pharmaceutical compounds to *Lemna gibba* using a seven-day static-renewal test. *Environmental Toxicology and Chemistry 23*(2), 371–382.

C.C.R.E.M. **(**Canadian Council of Resource and Environment Ministers). (1991). *Canadian Water Quality Guidelines Appendix IX*. Inland Water Directorate. Environment Canada, Ottawa, Canada. p. IX-1 to IX-8.

Carraschi, S.P., Shiogiri, N.S., Venturini, F.P., da Cruz, C., Girio, C.F.A., & Neto, J.G.M. (2011). Acute Toxicity and Environmental Risk of Oxytetracyline and Florfenicol antibiotics to Pacu (*Piaractus mesopotamicus*). *Bol. Inst. Pesca 37*(2), 115–122.

Finney, D.J. (1971). *Probit analysis*. 3rd Edition. Cambridge University Press. London, UK and New York.

Fridman, S., Sinai, T., & Zilberg, D. (2014). Efficacy of garlic based treatments against monogenean parasites infecting the guppy (*Poecilia reticulata* (Peters)). *Veterinary Parasitology 203*(1-2), 51-58.

Gupta, P.K., Khangarot, B.S., & Durve, V.S. (1981). The temperature dependence of the acute toxicity of copper to a fresh water pond snail, *Viviparus bengalensis* L. *Hydrobiologia 83*, 461-464.

Jahanjoo, V., Yahyavi, M., Akrami, R., & Bahri, A.H. (2018). Influence of Adding Garlic (*Allium sativum*), Ginger (*Zingiber officinale*), Thyme (*Thymus vulgaris*) and Their Combination on the Growth Performance, Haemato-Immunological Parameters and Disease Resistance to *Photobacterium damselae* in Sobaity Sea Bream (*Sparidentex hasta*) Fry. *Turkish Journal of Fisheries and Aquatic Sciences 18*, 633-645. DOI: 10.4194/1303-2712-v18_4_15.

Joseph, P.K., Rao, K.R., & Sundaresh, C.S. (1989): Toxic effects of garlic extract and garlic oil in rats. *Indian Journal of Experimental Biology 27*(11), 977-979.

Karber, G. (1931). Beitrag Zur Kollektiven Behandlung pharmakologischer Reihen versuche. Naunyn-Schmiedebergs Archiv fur Experimentelle Pathologie and Pharmakologie *162*, 480-483.

Kreutzmann, H.L. (1977). The effects of chloramphenicol and oxytetracycline on hematopoiesis in the European eel (*Anguilla anguilla*). *Aquaculture 10*, 323–334.

N.A.S./N.A.E. **(**National Academy of Sciences/National Academy of Engineering). (1973). *Committee on Water Quality Criteria*. US Government Printing Office, Washington DC, WQC. EPA-R-R3-033.

Ramadu, K.R., & Dash, G. (2013). A review on herbal drugs against harmful pathogens in aquaculture. *American Journal of Drug Discovery and Development 3*(4), 209-219.

Romero, J., Feijoo, C.G., & Paola, N. (2012). *Antibiotics in Aquaculture* – Use, Abuse and Alternatives, Health and Environment in Aquaculture, Dr. Edmir Carvalho (Ed.). doi: 10.5772/28157.