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Acute toxicity of Phenol on the vital organs of *Heteropneustes fossilis* showing different Enzymatic trends of AchE

Seema Dixit

Department of Zoology,
Sarojini Naidu Govt. Girls P.G. Autonomous College, Bhopal-462 016 (India)

Abstract

Phenol and its compounds cause inhibition of acetylcholinesterase activity in Brain and Kidney of *Heteropneustes fossilis* by increasing the Km and V_{max} values. They prove to be non competitive inhibitor with respect to AchE enzyme Kinetics. Rate of inhibition was more in Kidney.

Keywords – *Heteropneustes fossilis*, phenol AchE, non competitive.

Phenol and its compounds are very lethal pollutants discharged into the running water bodies from chemical industries such as coal refineries, pharmaceutical industries of textile dyeing paint leather petrochemicals[8,16,18]. Despite the active metobolism and detoxification of Phenol in Fish[12] sublethal doses of phenol interfere metabolism by affecting enzyme activities.

It is well known that AchE in animal tissues catalyzes the hydrolysis of Ach to choline and acetic acid at neuromuscular junction. Inhibition of AchE causes huge Ach accumulation which results in impaired metabolism, irregular transmission of nerve impulses and abnormal muscle coordination. Although lot is known about the seriousness of toxic compounds but the research information on the phenol toxicity is very less and lot of investigation is further needed.

The objective of the present study was to evaluate the acute toxicity of phenol on some vital organs of *H. fossilis* with respect to AchE Enzyme Kinetics and moreover this kinetic study could be used as an assertive tool to evaluate the toxicity of pollutants in animal tissues, there by helping to check the pollutant level and further promoting to adopt safety measures.

Heteropneustes fossilis healthy, normal and of standard size (8-12 cms.) were procured from Motia Talab and were kept in glass aquaria containing dechlorinated tap water. They were acclimitized to laboratory conditions for two weeks. They were fed daily with dried shrimp powder until two days prior to acute exposure of test toxicants Lc 50 value of phenol was determined by U.S. Std. graphical interpolation method (1976) and was estimated to be 30 mg/lt. Two sublethal

concentration of Lc 50 (*i.e.* safe doses) 1/5th and 2/3rd were taken i.e. 6 mg/lt and 20 mg/lt. Each groups of 10 fishes were exposed to two sublethal concentrations for 96 hrs. and control was kept and maintained parallel. At the end of experiment the control and experimental fishes were dissected and the organs, brain and kidney were removed. Tissue homogenate of 5% was prepared in ice cold 0.25 M sucrose solution at 12,000 rpm. AChE activity was measured spectrophotometrically at 540nm by Hestrin and Metcalf (1951) using ACHI as substrate. Protein estimation was done according to Lowry etal. (1951) using Bovine serum albumin as standard. The AchE activity Km, Vmax, percentage of inhibition and nature of inhibition at different concentration of toxicants were investigated by line Weaver Burk plot.

The present study on the Brain of *H. fossilis* shows value of K_m $0.83x10^{-3}$M which increased to $1.25x10^{-3}$M with 5 mg/lt. of phenol which finally reached to $1.42x10^{-3}$M with 20 mg/lt. phenol. The V_{max} of control Brain was 2.22 A/mg protein/30 min. which increased to 3.33 A/mg protein/30 min. and finally reached to 5.0 A/mg protein/30 min. The slope obtained from control (uninhibited enzyme) and dose concentration (inhibited enzyme) intercepted at different ordinates with respect of Michael's menten constant showing non competitive nature of inhibition. **Ref. to Tabe No. (1) and Fig. No. 1.**

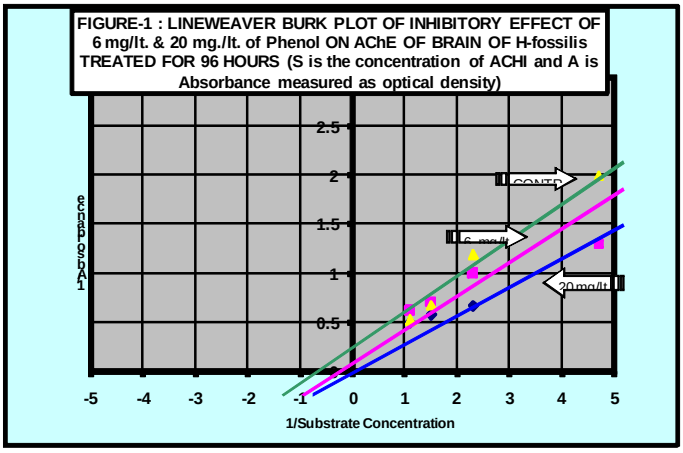

Table-1. Acute Effect of Different Concentrations of Phenol on Kinetic Parameters of Km & Vmax of Ache of Brain of *H.fossilis* (Substrate Used Was Achi)

Sl. No.	Phenol concentration in mg./lt.for 96 Hrs.	Kinetic parameters	
		Km x 10^{-3}M	Vmax (Absorbance) / mg. protein / 30 min.
1	CONTROL	0.83	2.22
2	6	1.25	3.33
3	20	1.42	5.00

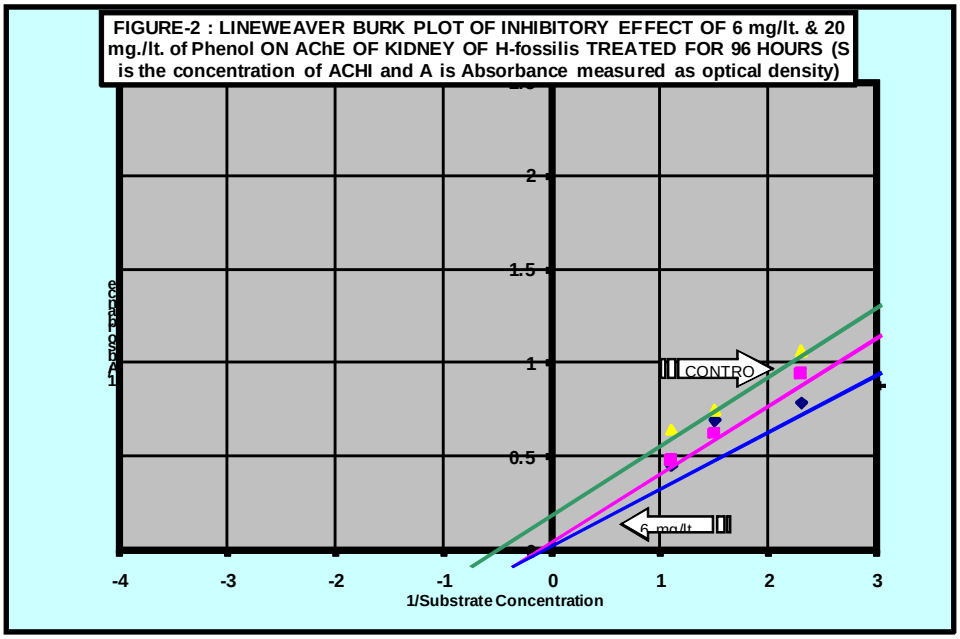

Table-2. Acute Effect of Different Concentrations of Phenol on Kinetic Parameters of Km & Vmax of Ache of Kidney of *H. fossilis* (Substrate Used Was Achi)

Sl. No.	Phenol Concentration in mg./lt.for 96 Hrs.	Kinetic Parameters	
		Km x 10^{-3}M	Vmax (Absorbance) / mg. protein / 30 min.
1	CONTROL	1.25	3.33
2	6	2.50	6.25
3	20	2.50	10.00

In Kidney of *H. fossilis* the control exhibited K_m vaue as $1.25x10^{-3}$M which increased to $2.5x10^{-3}$M in 6 mg/lt. phenol and remained constant i.e. $2.5x10^{-3}$M with 20mg/ lt. phenol concentration. While V_{max} in control showed a value of 3.33 A mg protein 30 Min. which increased to 6.25 A/mg protein/30 min. in 6 mg/lt. phenol and reached to maximum value of 10 A/mg protein/30 min. with 20mg/ lt. phenol concentration. The slope obtained from control and treated Kidney of *H. fossilis* intercepted at different ordinates with respect of Michael's menten constant, thus displaying non competitive nature of inhibition. **Ref. to Tabe No. (2) Fig No. (2)**

The present study in the vital organs of *H. fossilis* with phenol showed inhibitory nature of enzyme Kinetics of AChE. In Brain and Kidney K_m and V_{max} showed a gradual

increase through out except for Kidney where K_m showed a constant value of $2.5x10^{-3}$M at 6 mg/lt. and 20mg/lt. phenol concentrations. The continuous increase in the value of K_m and V_{max} indicates increasing level of AChE inhibition thus causing depletion in the hydrolytic power of enzyme and thereby stimulating continuous increase in the level of substrate molecule in the medium *i.e.* (ACh). The intersection of calibration curves at different ordinates with respect to Michel's mention constant in control and treated groups clearly shows non competitive nature of inhibition and phenol to be a neurotoxic and non competitive inhibitor with respect to the enzyme Kinetics of AChE in *H. fossilis*. This is well supported by the findings of Carter *et al.*[4], Coppage *et al.*,[6], Rao *et al.*,[19], Bashamohideen and Sailbala[2], Hande and Pradhan[11], Balasubramanian and Ramaswami[1], Devraj *et al.*,[7], Thangavel and Ramaswamy[21], Tembhre and Kumar[20], Farhina and Singh[10], who have also reported. Similar trends of non competitive nature of inhibition in different Fish species with different group of toxicants.

In the phenol category, in both tissues the continuous increase in the values of K_m and V_{max} are seen which indicates increasing level of AChE inhibition thus causing depletion in the hydrolytic power of enzyme and there by stimulating continuous deposition of substrate ACh molecules. The intersection of calibration curves at different ordinates in control and treated group of all tissues clearly show non competitive nature of inhibition and phenol to be a neurotoxic and non competitive inhibitor with respect to enzyme Kinetics of AChE in *H. fossilis.*

With reference to automobile fuels (Phenol) toxicity percentage inhibition (K_m) was maximum in kidney followed by brain **(K_m Value** kidney > brain) while $\mathbf{V_{max}}$ was maximum in kidney followed by brain **(V_{max} value** kidney > brain).

References :

1. Balasubramanian, S. and M. Ramaswami (1991). Jour. Ecobiol., *3*(2) : 117-132.
2. Bashamohideen, M. D. and S. Ramanna Devis (1989). Behavioral changes on symptoms of poisoning in Indian major carp (Labeo rohita subjected to Phosphamidon exposure). Proc. 61th Indian Sci. Long. Abst. 1.
3. Cappon C.J. (1987). *Bull. Environ. Contam. Toxicol. 38 :* 695 – 699.
4. Carter (1971). In vie studies of brain acetylcholinesterase inhibition by organophosphae and Carbamate insecticides in fish Ph.D., dissertation Louisiana State University, Baton Rough, Louisiana.
5. Chandravathy, V. Mary and S.L.N. Reddy (1996). *J. Environ. Biol. 17*(1): 75-79.
6. Coppage, D.L., E. Mathew's, G.H. Cook and J. Knight (1974). *Biochem. and Physiol., 5:* 536-542.
7. Devaraj, P.S., Durairaj and V.R. Selvarajan (1992). *J. Ecotoxicol. Environ. Monit., 2*(2) : 99-103.
8. Doubale D.D., S.A. Barra Glough, R.M. Bean and W.E. Fallon (1983) *Trans. Am. Fish Soc., 112:* 712-719.
9. Dulka, J.J. and T.H. Risby (1976). Ultratrace metal in some environmental and biological system. *Anal. chem. 48 :* 640-653.
10. Farhina, Pasha and Romsha Singh (2005). *Asian Jour. Exp. Sci., 19*(2) : 119-126.
11. Hande, R. A. and P. V. Pradhan (1990). *Proc. Ind. Acad. Sci. (Anim. Sci.), 99*(1):

53-56.

12. Hoarand, Randall (1969) – Academic Press London Reproduction in Fish Physiology.
13. Jhingan, V.G. (1985). Fisheries and water pollution in Varshney, C.K. (Ed.) Water Pollution and Management, New Delhi (India) Wiley Eastern 166 – 186.
14. Konar S.K. (1979). Pesticides and aquatic ecosystem, *Indian Jour. Fish, 22 :* 80 – 85.
15. Kumaraguru, A.K. (1995). *Ecol. Environ. Cons., 1*(1-5) : 143-150.
16. Kumaran P. (1993)– Specialized microbes in Phenolic Waste Management *J. IAEM 20:* 15-20.
17. Lowry, O.H.; N.J. Rosenbrough, A.L. Farr and R.J. Randall (1951). *Jour. Biol. Chem., 193 :* 265-275.
18. Mukherjee D., S. Bhattacharya, V. Kumar, and J. Moitra (1990) *Biomed. Environ. Sci. 3:* 337-442.
19. Rao, I.M., R. Manjula and Sree Patnaik, (1997). *Indian J. Fish, 44*(4) : 405-408.
20. Tembhre, M. and S. Kumar (1995). *Indian J. Spect., 6(2) :* 39-42.
21. Thangavel, P. and M. Ramaswamy (1992). *Ind. J. Exp. Physiol., 12*(11) : 824-827.