

Inhibitory Chronic Effect of Copper Sulphate on Acetylcholinesterase Activity and Enzyme Kinetics with Its Subsequent Reactivation in the Stomach of *Rattus norvegicus*

Urmila Saluja* and Santosh Kumar**

Department of Zoology and Aquaculture
Govt. P.G. College, Sehore 466 001 M.P.

Abstract : Study was conducted to find out the chronic toxicity of sublethal concentration of copper sulphate on the activity of AChE and its kinetics in the stomach of *Rattus norvegicus*. The inhibition of AChE activity was time dependent and the pattern of inhibition of AChE activity due to copper sulphate was mixed *i.e.*, competitive- noncompetitive. The AChE activity was slowly reactivated to almost normal after 15 days of treatment.

Key words : AChE, Inhibition, Recovery, Km, Vmax, *Rattus norvegicus*.

Introduction :

Copper is widely used as food and fertilizer additive, in insecticide mixture, as mordant in textile dyeing, in tanning leather, in preserving wood, in laundry, pigment in paints, varnishes, tonner in photography, etc. (The Merch Index, 1983). Thus, its widespread usage increased the scope of exposure of man to this heavy metal. The harmful effect of heavy metals on the physiology, biochemistry and enzyme system of animals has been studied by many workers (Weiss and Simon, 1975; Cooper and Manalis, 1982; Sinha, *et al*; 1992; Tembhre and Kumar, 1995, Margarat and Jagdeesan, 1999).

Keeping this into the consideration the present study was planned to evaluate the chronic toxic effect of copper on the specific activity of AChE and its kinetics in the stomach of *Rattus norvegicus*. The withdrawal effect of copper has also been worked out to observe the recovery of AChE activity.

Materials and Methods :

Healthy specimens of *Rattus norvegicus* weighing 100 ± 5.0 gms were collected and acclimatized to the laboratory conditions for 10 days. The oral LD₅₀ of copper sulphate for 96 hrs. was determined as 900 mg/kg body weight by plotting the dose mortality curve as suggested by Trevan (1927). The rats

* Corresponding Author.

** Former Vice Chancellor Dr. Hari Singh Gaur University, Sagar (M.P.)

Saluja U. and Kumar S. (2005) *Asian J. Exp. Sci.*, 19(1), 65-71

were given oral sublethal dose of Copper Sulphate ($1/7^{\text{th}}$ of LD_{50} *i.e.*) 128.57 mg/kg body weight) on every day for 15 and 30 days. After the treatment of 15th days the rats were allowed for recovery of AChE activity by cessation of pesticide for next 15 days. Rats were sacrificed by cervical dislocation and stomach was taken out and homogenised in 0.25 M sucrose solution followed by centrifugation. Colorimetric method of Metcalf (1951) was used for the determination of AChE activity using AChI as a substrate. Protein contents were determined by the method of Lowry *et al.*(1951). Enzyme kinetics was studied by calculating K_m and V_{max} by applying Line-Weaver Burk plot. The student's "t" test (Ipsen and Feigel, 1970) was used to calculate the statistical significance between control and experimental values.

Result and Discussion :

In the present study, the treatment of sublethal dose of copper sulphate has raised the K_m to 2.49×10^{-3} M after 15th days and 3.04×10^{-3} M after 30 days from the control value of 1.65×10^{-3} M. Whereas, the V_{max} was found to be decreased from the control value of 1.03 A /mg protein /30 min to 0.86 and 0.7A/ mg protein/30 min after 15 days and 30 days, respectively (Table-1). After applying Line - Weavers Burk plot the corresponding increased value of K_m and decreased V_{max} proves that the copper sulphate produces mixed inhibition of AChE (Fig. 1). Copper sulphate inhibit the AChE activity in a Mixed way *i.e.*, competitive and non competitives, has been reported by many workers (Nemscsok *et al.*,1984; Nemscsok *et al.*,1990; Tembhre and Kumar, 1995)

AChE is the target of a number of toxicants (Siddiqui *et al.*, 1991) AChE inhibition probably causes death in higher vertebrates by blocking neurotransmission in the respiratory centre of brain and in the neuromuscular junctions (Nemscsok *et al.*,1990). The increase in ACh level has been attributed to both presynaptic and synaptic cleft accumulation of the neurotransmitter presumably due to AChE inhibition (Margarat and Jagdeesan, 1999).

Results of the present study show a reducing trend of AChE activity in a time dependent manner at sublethal concentration of copper sulphate (Table - 1). There was 26.31% inhibition of AChE activity on 15th day, which

Table1 : Acetylcholinesterase activity, ACh content, K_m , and V_{max} of stomach of normal and 128.57 mg/kg body weight of copper sulphate treated *Rattus norvegicus* after 15 and 30 days exposure and also after 15 days recovery.

Parameters	Control / Duration of Exposure / Recovery			
	Control	15 days	30 days	Recovery (15 days)
AChE specific activity (μ moles of ACh Hydrolysed/ mg protein /hour)	1.498 + 0.086	1.104*** \pm 0.066	0.867*** \pm 0.114	1.377 \pm 0.068
% Inhibition	—	26.31	42.13	8.08
ACh Content (μ moles / gm wet weight of tissue)	90.325 \pm 4.819	116.302 \pm 4.473	126.987*** \pm 5.735	101.317*** \pm 2.048
% Increase	—	28.75	40.58	12.16
$K_m \times 10^{-3}$ M	1.87 \pm 0.153	2.71*** \pm 0.277	3.04*** \pm 0.319	2.34* \pm 0.372
V_{max} (A/mg protein / 30 min)	1.0 \pm 0.05	0.74*** \pm 0.07	0.61*** \pm 0.055	0.9* \pm 0.065

Values are expressed as mean S.D. of five individual observations. P values * P < 0.05, ** P < 0.01, *** P < 0.001.

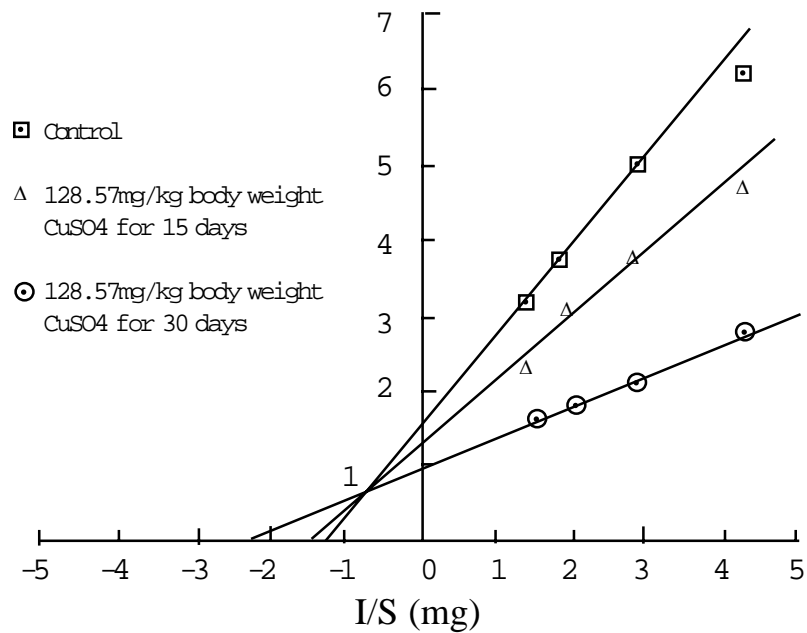


Fig. 1 : Line weaver - Burk plot of the inhibition of AChE of stomach of *Rattus norvegicus* by copper sulphate for 15 and 30 days.

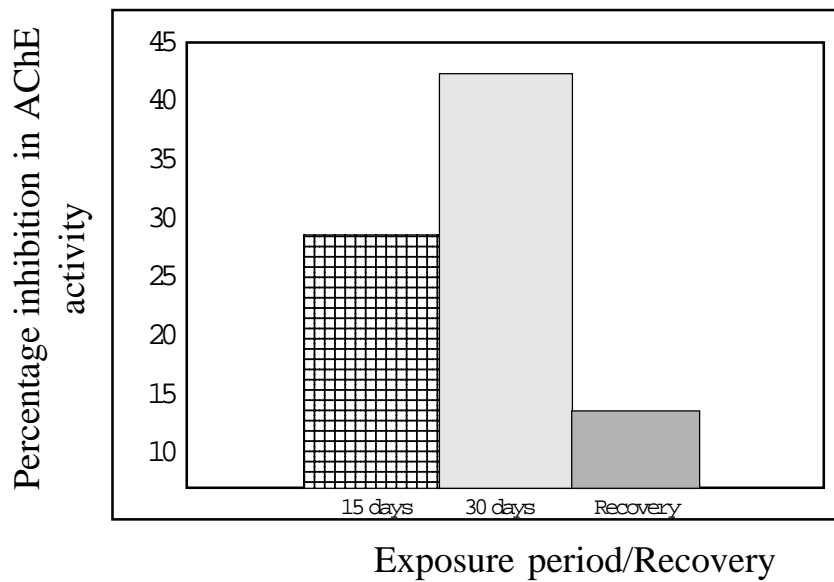


Fig. 2 : Percent inhibition of AChE activity in the stomach of *Rattus norvegicus* treated with 128.57 mg/kg body weight of copper sulphate for 15 and 30 days and after 15 days recovery.

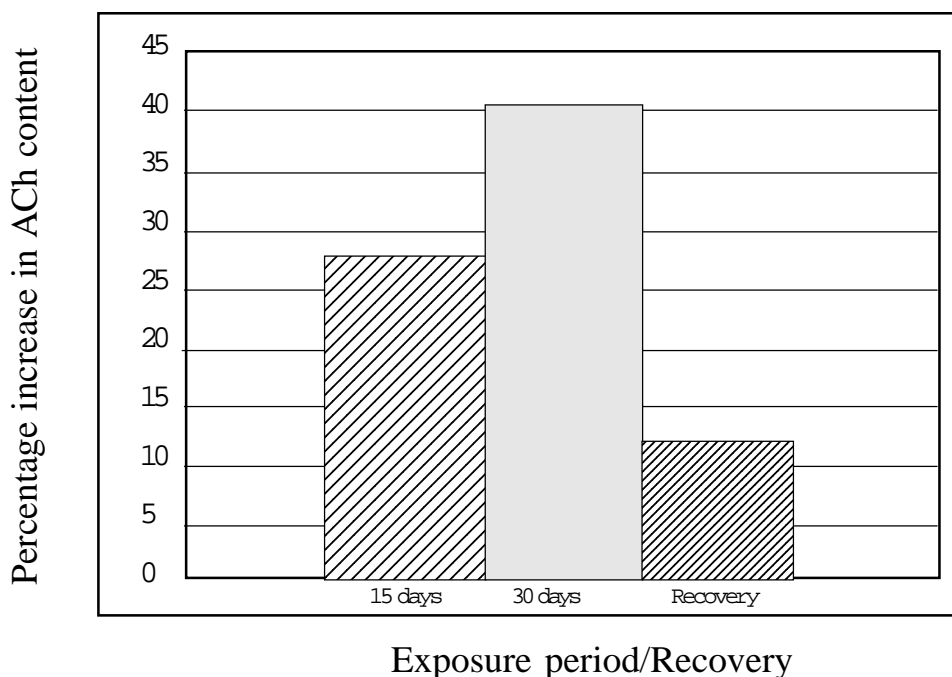


Fig. 3 : Percent increase in ACh content in the stomach of *Rattus norvegicus* treated with 128.57 mg/kg body weight of copper sulphate for 15 and 30 days and after 15 days recovery.

increased to 42.13% on 30th day of exposure to 128.57 mg/kg body weight of copper sulphate (Fig. 2). Whereas, ACh content was increased to 28.75% and 40.58% on 15th and 30th day, respectively (Fig. 3). Copper was also reported to act as a neuromuscular blocking agent (Tembhre and Kumar, 1995) lending support to our observations which can be correlated to the results of the present study.

Results of 15 days recovery study after the intoxication for 15 days, reveal that AChE activity returned to almost normal *i.e.* 1.377 μ M of ACh hydrolysed/mg protein/hour (Fig. 4) indicating that the effect of copper on AChE of stomach was temporary and recovered quickly when the toxic stress is removed.

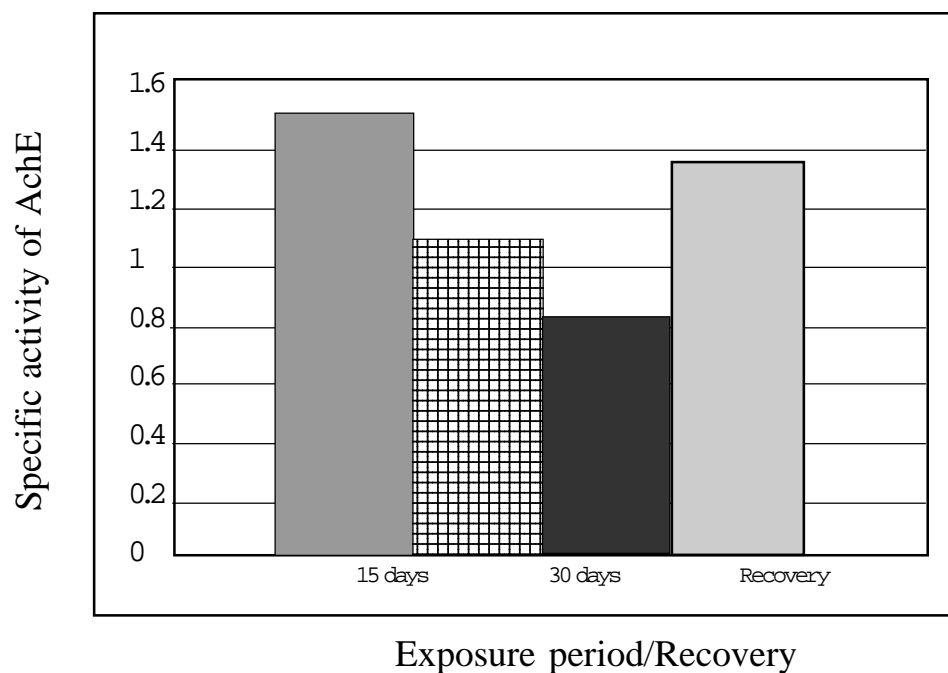


Fig. 4 : Specific activity of AChE of stomach of *Rattus norvegicus* treated with 128.57 mg/kg body weight of copper sulphate for 15 and 30 days and after recovery. The Specific activity is expressed as 1 moles of ACh hydrolysed/ mg protein/ hour.

References :-

- Cooper G.P. and Manalis R.S. (1982) : Influence of heavy metal on synaptic transmission. *Neurotoxicol. Review* **4(4)**, 69-88
- Ipsen J. and Feigel P. (1970) : In Bancrofts Introduction to Biostatistics, 2nd Ed. Harper and Row Publishers. Inc.; New York.
- Lowry O.H., Rosen brough N. J., Farr A.L. and Randall R.J. (1951) : Protein measurement with folinphenol reagent. *J. Biol. Chem.* **193**, 265-275.
- Margarat A. and Jagdeesan G. (1999) : Effect of mercuric chloride and the influence of penicilliamine on acetylcholinesterase (AChE) activity in mice. *Poll. Res.* **18(3)**, 293-296.
- Metcalf R.L. (1951) : In Methods in Biochemical Analysis (D. Glick ed.). *Interscience Publishers Inc., New York. 1.*

Copper Sulphate on Acetylcholinesterase Activity

Nemcsok J., Nemeth A., BYZAS Zs. and Bross L. (1984) : Effects of Copper, Zinc and Paraquat on AChE activity in Carp. *Aquatic Toxicol*; **5(1)**, 23-31.

Nemcsok J., Asztalos B. and Szabo A. (1990) : The effect of methidathion, paraquat and copper sulphate singly or in combination on AChE activity of carp. *Biol. Monitoring of Environ. Poll.* (eds. M. Yasuno and Whitton, B.A.) Tokai Univ. Press.

Siddiqui M.K.H., Rahman M.F., Mustafa M. and Bhalerao U.T. (1991) : A Comparative study of blood changes and brain acetylcholinesterase inhibition by monocrotophos and its analogues in rats. *Ecotoxicol. Environ. Saf*; **21**, 283- 289.

Sinha G.M., Kesh A.B., Sengupta K. and Das A.K. (1992) : Studies on the cadmium intoxication and the action of antagonists on the intestine of an Indian air breathing fish, *Anabas testudineus* (Bloch). *J. Fresh water Biol.* **4 (4)**, 273-281.

Tembhre M. and Kumar S. (1995) : Acetylcholinesterase activity and enzyme kinetics in the gut of *Cyprinus carpio* subjected to acute and chronic exposure to copper. *J. Ecobiol.*, **7(3)**, 191-195.

The Merck Index (1983) : *Tenth Edition, Merck and Co; Inc. Rahway, N.J., U.S.A.*

Trevan J.W. (1927) : Proc. R. Soc. ; London, **101**, 483, By Pascoe, D. (1983) : In "Toxicology". *Acute, Chronic and special toxicity tests. Edward Arnold, London* : 44- 50.

Weiss B. and Simon W. (1975) : Long-term toxicity of methyl mercury. In *Behavioural Toxicology. Edited by B. weiss, V.G. Laties, Plenum press, New York*, PP. 429-439.