

## **Effect of EDTA on the Haemolymph, Fatbody and Ovary Amino Acid and protein Concentration in Immature *Cybister confusus* Sharp**

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**Abstract:** Effect of Ethylene Diamine Tetra Acetic acid (EDTA) has been observed in haemolymph, fat body and ovary protein and amino acid concentration in immature *Cybister Confusus* Sharp. A realistic significant fall of concentration in protein has been observed in haemolymph and fat body. Whereas in ovary EDTA has almost no effect on protein concentration. Finding is further supported by rise of amino acid concentration in haemolymph fat body and almost no effects on ovary free amino acids. Probably EDTA apparently does not act at molecular level to alter the protein synthesizing machinery of the immature insects but break down the proteins into amino acids to supply the energy to the insects.

### INTRODUCTION

A large number of works have been carried out by numerous workers on fishes, aquatic insects, plants, terrestrial insects and mammals under different conditions. Most of the workers have reported alternation in normal biochemical constituents in the body of the organism. Wyatt (1961) found deviation from normal biochemical constituents in insect haemolymph, Islam and Roy (1982;83), in *Schizodactylus monstrosus*, Roy et al. (1983) in *Schizodactylus monstrosus*, Phukon et al. (1986) in *Hydrophelous olivaceous*, Kumari and Naidu (1987) in *Bombyx bori*, Rajani et al. (1988) in the tissue of freshwater prawn, *Caridina rajadhari*, Satya Sharma and Ehteshamuddin, (1988) in *Sphaerodema rusticus*, Rani et al. (1991) in *Hydrophilous olivaceous* Fab. Khan et al. (1998) in *Cybister confusus*, Niranjan Jan Choudhary et al. (1998) in *Hydrophelous olivaceous*. However, only little information has been offered with regard to concentration of protein and amino acids under various circumstances with EDTA treatment in immature insects.

## Materials and Methods

The immature *Cybister confuses* were collected from the local ponds. Insects were brought to laboratory in class aquaria. Some fish, hyacinth and Hydrilla were kept inside the aquaria for a week for acclimatization prior to perform experiment.

The reared insects were divided in three different groups. One reared in normal laboratory condition without application of any chemical and served as control. The other was exposed to the sub lethal dose of EDTA (0.0025%). Free amino acid and Protein estimation were done after the treatment of 24, 48, 72 hrs and seven days of treatment.

Quantitative estimation of haemolymph, fat body, ovary free amino acids and protein of immature insects were done by Moore and Stein (1954) and Lowry's method (1951) respectively.

## Amino Acid

### Haemolymph

Free Amino Acid Concentration in Haemolymph of immature untreated insects was noted  $25 \pm 4$   $\mu\text{mg/ml}$ , EDTA treatment has an Amino acid elevating effect significantly that is  $120 \pm 12$   $\mu\text{mg/ml}$  ( $P < 0.001$ ) at 24 hrs, declining slightly to  $96 \pm 10$   $\mu\text{mg/ml}$  at 48 hrs, still remaining higher than that of untreated insects i.e.  $80 \pm 8$   $\mu\text{mg/ml}$  at 72 hrs and  $32 \pm 6$   $\mu\text{mg/ml}$  at 7 days of treatment (Table-1, Fig.1).

### Fatbody

Free Amino Acid Concentration in fat body of immature untreated insects was noted  $28 \pm 6$   $\mu\text{mg/ml}$ , on treatment With EDTA the free amino acid concentration decline gradually to  $16 \pm 2$   $\mu\text{mg/ml}$  at 24 hrs,  $16 \pm 3$   $\mu\text{mg/ml}$  at 48 hrs,  $8 \pm 1$   $\mu\text{mg/ml}$  at 72 hrs and  $8 \pm 1.5$   $\mu\text{mg/ml}$  ( $P < 0.01$ ) at 7 days (Table-1, Fig.1).

### Ovary

Free Amino Acid Concentration in ovary of immature untreated insects was  $1.6 \pm 0.2$   $\mu\text{mg/ml}$ , Treatment with EDTA no significant changes have been observed that is  $0.9 \pm 0.1$   $\mu\text{mg/ml}$  at 24 hrs,  $1 \pm 0.1$   $\mu\text{mg/ml}$  at 48 hrs,  $1 \pm 0.2$   $\mu\text{mg/ml}$  at 72 hrs,  $1.6 \pm 0.4$   $\mu\text{mg/ml}$  at 7 days of treatment (Table-1, Fig.1).

## Proteins

### Haemolymph

Protein concentration in Untreated i.e. controlledimmature female insectwas noted121.2±8 µmg/ml, EDTA has a protein lowering effect in haemolymph of immature female insects i.e. decline to 55±5 µmg/ml(P<0.001) at24 hrs, 60±5.6 µmg/ml(P<0.001) at48 hrs, 70.5±5.8 µmg/ml (P<0.001) at 72hrs and 35.4±3.5 µmg/ml (P<0.001) at 7days of treatment(Table-2, Fig.2)

### Fat body

Protein concentration in fatbody of immature insects under controlled condition was reported83±5 µmg/ml ,EDTA treatment has protein declining effect (p<0.05) that reduced to 60.5±4.5 µmg/ml at 24 hrs and 60.8±4 µmg/ml at 48hrs, it rose slightly but insignificantly(p>0.05)to 70.5±5 µmg/ml at 72hrs and 85.5±6.5µmg/mlat 7days of treatment(Table-2, Fig.2)

### Ovary

Concentration ofproteins in ovary of untreated immature insect was noted 9.09±1.5 µmg/ml, on treatment with EDTAprotein concentration decline to 6.5±1.5 µmg/mlsignificantly (P<05)at 24 hrs, butrose insignificantly (p>0.05) to 7.6±1 µmg/ml at48hrs, 8.5±2 µmg/ml at72hrs and 7.5±1.5 µmg/ml at 7days of treatment(Table-2, Fig.2).

### Discussion and Conclusion

Decline in the protein concentration and rise in the amino acid concentration has been described byIslam and Roy (1982),Roy et at.(1983), in *Schizodactylus monstrosous*, Kumari and Naidu( 1978) ,describe the effect of Permmethrin on *Bombyx bori* and found that total protein concentration of the insects decrease significantly after the treatment with the chemical.Khan et al.(1998) reported the declining effect of DDT and EDTA in carbohydrates in the body of immature and Mature *C.confusus* female.Choudhary,et al.(1998) describe the Effect of Copper Sulfate on protein concentration in aquatic Bettle *Hydrophilous olivaceous*(Hydrophilidae,Coleoptera) and reported protein declining effect

.In present investigation,EDTA treatment resulted into a sharp decline in the free amino acids in haemolymph of immature female insects at 24 hrs than declined gradually and come up almost to the normal level after 7days of treatment. Fatbody free amino acid concentration in the immature female insects has gradually declining effect after treatment with the chemical. Whereas on protein concentration of immature ovary the chemical almost has no effect .EDTA treatment resulted decline in haemolymph protein concentration upto 7days. An early but slightly declining effect has been observed in the protein concentration

of fatbody of immature female insects .There is almost no effect of EDTA on ovary of immature insects.

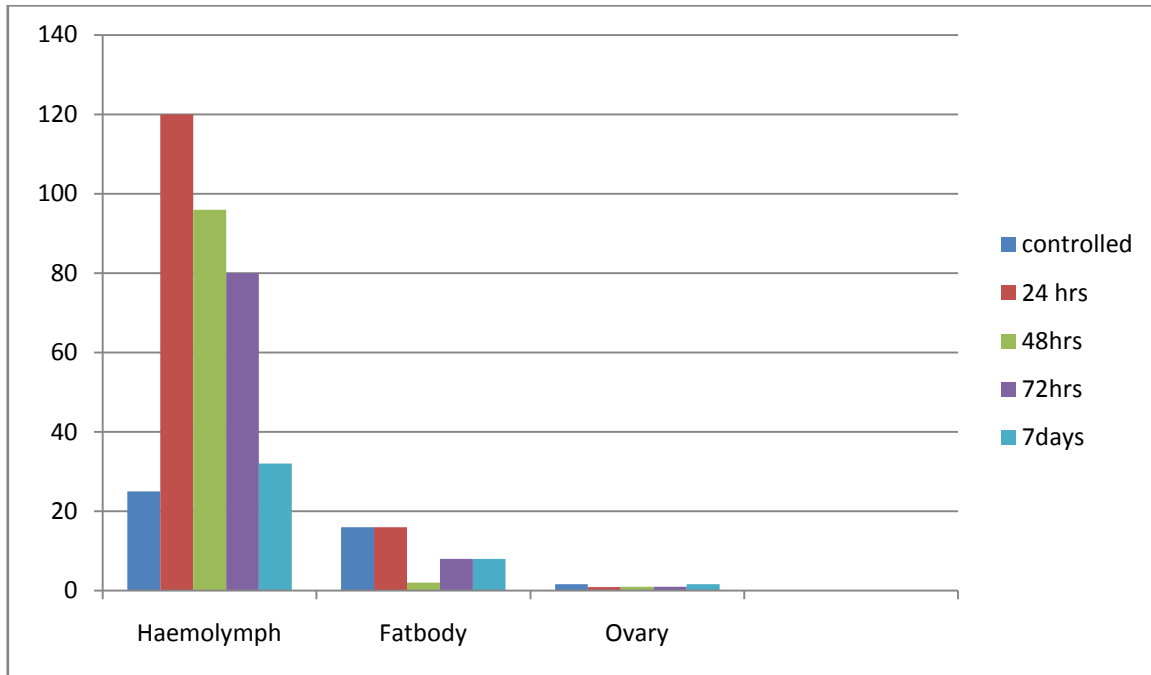
It can be concluded that EDTA apparently either did not have any adverse effect on the synthesis of proteins. A decline in haemolymph protein concentration and an early increase and later decline in thehaemolymph free amino acids concentration of the immature insects can be attributed to the fact that due to non availability of main food stuff i.e. carbohydrate, as EDTA has hypoglycemic effects, protein breakdown into amino acids to liberate energy for metabolic activity.

Constituent	Controlled Concentration	Treated Concentration			
		24 hrs	48hrs	72 hrs	7days
Haemolymph	25±4µmg/ml	120±12µmg/ml	96±10µmg/ml	80±8 µmg/ml	32±6µmg/ml
Fatbody	28±6µmg/ml	16±2µmg/ml	16±3µmg/ml	8±1µmg/ml	8±1.5µmg/ml
Ovary	1.6±.2µmg/ml	.9±.1µmg/ml	1±.1 µmg/ml	1±.2 µmg/ml	1.6±.4µmg/ml

Table-1 : Amino acids concentration in controlled and EDTA Treated C. confuses female

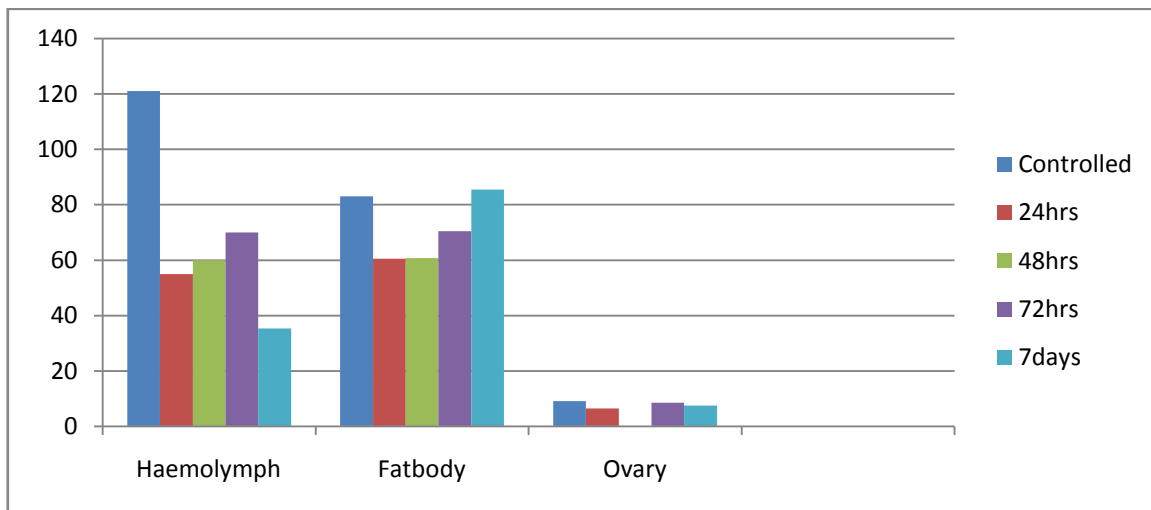
Constituent	Controlled Concentration	Treated Concentration			
		24 hrs	48hrs	72 hrs	7days
Haemolymph	121.2±8 µmg/ml	55±5 µmg/ml	60±5.6 µmg/ml	70.5±5.8 µmg/ml	35.4±3.5 µmg/ml
Fatbody	83±5 µmg/ml	60.5±4.5 µmg/ml	60.8±4 µmg/ml	70.5±5 µmg/ml	85.5±6.5 µmg/ml
Ovary	9.09±1.5 µmg/ml	6.5±1.5 µmg/ml	8.5±1 µmg/ml	8.5±2 µmg/ml	7.5±1.5 µmg/ml

Table-2: Protein concentration in controlled and EDTA Treated C. confuses female



**Figure-1,** Concentration of free amino acids in µg/ml in Haemolymph, Fatbody and Ovary of immature

*C. confuses*



**Figure-2,** Concentration of proteins in µg/ml in Haemolymph, Fatbody and Ovary of immature

## References

1. Islam,A. and Roy,S(1982) Free amino acids in the gut, fatbody,gonads and haemolymph of *Schizodactylus monstrosous*, Drury(orthoptera;Schizodactylidae).Possible role of intra cellular bacteria.*Proc.Nat.Acad. Scien.*48: 26-30.
2. Islam,A. and Roy,S (1983).Variation of free amino acids during flight in*Schizodactylus monstrosous**Curr. Sci.*52 79-83.
3. Kumari, P. and B.P. Naidu(1987) Study on the effect of Paramethrin on organic constituents of *Bombyx bori*. *Environment and Ecol.*5(4) 417-419
4. Lowery,D.H.,Rosenbrough,N.J.,Farr,A.L and Randal,R.J.(1951) protein measurement with the phenol reagent.*J.Bio.Chem.*193: 265-270
5. Moor,S and Stein,W.H.(1954) A modified ninhydrin reagentfor photometric determination of amino acids and related compounds. *J.Bio.chem.*211: 907-913
6. Phukon,L and Prasad B.(1986) A note on the Qualitative estimation of free amino acids in the haemolymph of *Hydrophilous olivaceous* Fab(Hydrophilidae,Coleoptera),*Com.Physio.Eco.*11(3) 123-126
7. Rajani,J.,Sarojini,S and Persis,B(1989) Alternation in total Protein Content and free amino acid level in fresh water prawn,Caridina rajadhani during starvation.*Cop physiol.Ecol.*14(4) 185-193
8. Rani Shrivastava,Satya Sharma.Uma nath saha and S Ehteshamuddin(1991) protein, amino acids and glycogen in the haemolymph and fatbody of *Hydrophelous olivaceous* Fab.(Hydrophilidae, Coleoptera), in relation to Starvation.*J.Freshwater Bio.*3(4) 295-298
9. Roy,S.L,Mandal,I.N and Islam, A.(1983) Free amino acids in haemolymph and reproductive organs of male *Schizodactylus monstrosous*, *Sci.and Culture.*49 :59-60
10. Satya Sharma and Ehteshamuddin(1988) Protein concentration in immature and adult*Sphaerodema rusticus*(Belostomidae Hemiptera)*Environ. and Ecol.*6(30) 765-767
11. Wyatt,G.R. !961) The biochemistry of insect haemolymph.*Ann.Rew.Entomol.*675-102
12. Choudhary,N.,Kumari,C.,Khan,S.,and Ehteshamuddin,S(1998) Effect of Copper Sulfate on protein concentration in aquatic Beetle *Hydrophilous olivaceous*(Hydrophilidae,Coleoptera) *Environ. and Ecol.*16(3) 573-578
13. Khan,S., Choudhary,N., kumara,C., and Ehteshmuddin,S,(1998) Effect of DDT on the haemolymph fatbody and ovary protein and amino acids concentration in Mature *Cybister confusus*(Dytiscidae Coleoptera). *J.Freshwater Bio.*10(1-2) 59-62