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Effects of Vit-C on the activities of Acetylcholine esterase and aminotransferases in Dimecron intoxicated developing chick embryos

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Abstract

When organophosphate insecticide, dimecron introduced into the fertilized hen's egg at a certain dose before incubation it shows a characteristic and interesting feature which has been studied in different developmental stages. Present study shows the ameliorating effect of Vit-C in different days of study schedule in the enzymatic activities of Acetylcholine esterase and aminotransferases.

Keywords: Acetylcholine esterase, alanine aminotransferase, atropine, dimecron, Vit-C.

Introduction

Fertilized avian egg is a highly organized system containing all essential food and reserves for its normal development. The pesticide residue directly disturbs the normal development and causes pathophysiological and morphological changes (Varnagy, 1992). Organophosphorus pesticides are associated with toxic response and toxicity is reflected by change in metabolic enzymes. Campbell and Ofurum (1986) reported changes in the activities of serum and liver enzymes following a short-term exposure to rat. The GOT activity was also higher than GPT when treated with organochlorine insecticide dieldrin at the dose level 0.25 of LD_{50} at different time intervals of rat brain (Ahmed et al., 1986). However, Synthetic pyrethroid insecticides decreased GOT activity in brain, but GPT activity has been increased in liver and heart, as well as GOT activity in heart (Majumdar et al., 1994). However, serum glutamate pyruvate transaminase and serum glutamate oxaloacetate transaminase activities showed irregular change which includes mild decrease or increase due to the exposure of an organophosphate insecticide in rabbits (Sawas, 1998).

The organophosphate pesticides are neurotoxic and acts as an inhibitor of AChE activity (Clegg and van Gemert, 1999; Jett et al., 1999). A significant acetylcholinesterase inhibition was noted in beagle dogs fed with chlorpyrifos in different doses in the diet (Mattsson et al., 2001). Ahmed *et al*. (1986) tested dieldrin for some enzymes including GOT and GPT as well as cholinesterase and showed that dieldrin caused a significant increase and the value of GOT was higher than GPT where as

cholinesterase activity was inhibited thoroughly during experimental period. Pena Llopis et al., (2003 a, b) in an experiment with dichlorvos intoxicated eels (*Anguilla anguilla*) showed an inhibition of AchE activity in brain. Hoffman and Sileo (1984) also observed the inhibition of brain AchE activity on Mallard development starting from 11 days to hatching. Whereas Misawa et al., (1981) observed inhibition of AchE activity about 90% at day six to eight.

Methodology

Fertilized eggs of Rhode Island Reds *(Gallus gallus*) were obtained from the Government poultry farm. Eggs were incubated at 37^0C $\pm$ 0.5^0C with an average relative humidity of 75% in the incubator. Dimecron, was used for the present study. Technical grade Dimecron (85% SL purity) was available from Hindustan Ciba-Geigy Limited, India. Bovine serum albumin (BSA), sodium dodecyl sulphate (SDS), DTNB, ATchI, EDTA and Vit-C (L-ascorbic acid) were purchased from Sisco Research Laboratory, India. AST and ALT test kit were purchased from Stanbio, India.

Preincubated eggs were injected with desired doses of insecticide according to the method of McLaughlin et al., (1963). The dose was selected as per the method followed by Sahu and Ghatak (2002). Ameliorating agent Vit-C and Atropine doses are selected as per schedule.

Study Schedule

Experiments were conducted both on control and treated individuals with following groups.

Group-I: Control,

Group- II: Dimecron treated

Group-III: Dimecron + Vit-C

Group-IV: Dimecron + Vit-C + atropine

The enzymatic study from liver was done on 8^{th} and 14^{th} day embryos while 4^{th}, 8^{th} and 14^{th} day embryos were selected for study in brain.

A definite amount of brain and liver tissue were taken and homogenized in 0.9% sodium chloride. The homogenate was centrifuged at 4000 $\times$g for 10 minutes at 4^0C. The supernatant was quantitatively collected and was used for further analysis. Acetylcholinesterase activity (AChE) in brain was determined by Ellman et al*.,* (1961). Aspartate aminotransferase (AST) activity in liver was measured by using AST test kit (Stanbio, India). The kit consisted of substrate for AST, DNPH solution, sodium hydroxide solution, Phosphate buffer solution and pyruvate powder for standard. Liver Alanine aminotransferase (ALT) activity was also measured by using ALT test kit (Stanbio, India). The kit consisted of substrate for ALT, DNPH solution, sodium hydroxide solution, phosphate buffer solution and pyruvate powder for standard.

Result

Acetylcholinesterase activity in brain (AchE)

The amount of choline derived from brain tissue at room temperature in the differentiating brain of chick embryos are not same. The activity of the enzyme as evidence by the presence of choline is shown in Table 1 and Fig. 1. It may observed that the activity of the enzyme varies during the study period. The specific activity of AChE is expressed as nmol of substrate hydrolysed min^{-1} mg $protein^{-1}$. In control group, the activity of AChE in brain of 8^{th} day embryo increases from its initial condition followed by further increment in the activity in 14^{th} day of incubation.

The pesticide exposure increases the AChE activity in brain so far as the different developmental period is concerned. But the activity decreases on all days the days under study compared to control. It is found that the activity of enzyme shows maximum (70%) reduction compared to control on 14^{th} days followed by 60% and 48% on 8^{th} and 4^{th} day of

incubation. When the differences of the data between the control and treated embryos are compared, it is seen that the difference is significant at both $p<0.05$ and $p<0.01$ level in 't-test. When Vit-C was administered against phosphamidon intoxicated embryos, the activity of AChE enzyme reaches nearer to control values i.e. Vit-C has ameliorating effect on enzyme AChE activity against intoxication. Statistically significant variation ($p<0.05$) is observed. In the Group –IV, when the recovery agent and atropine are administered against the phosphamidon intoxicated embryos; it is observed that Vit-C shows closer data with the Group-III embryos. It shows that atropine treated groups show lesser activity than the recovery agent group.

Aspartate Aminotransferase (AST) activity in Liver

The AST activity from liver of developing chick embryos has been presented in Table 2 and Fig. 2. It is observed from the table that the activity of the enzyme is not same and varies during the study period. The activity of AST in group I increased from 8^{th} day to 14^{th} day chick embryos. Thus, AST activity of 1.64 I.U/L was obtained on 8^{th} day and 2.17 U/L of development.

In dimecron treated groups, AST activity increased in both the days under study. Thus the activities observed as 2.82 and 3.56 U/L in 8^{th} and 14^{th} day embryos respectively. The variation of the activity of the enzyme between the control and treated was statistically significant ($p<0.05$).The variation of the activity of the enzyme between the control and treated was statistically significant ($p<0.05$). Vit-C treated embryos AST activity recovered nearer to the normal in comparison to the treatment groups in both the days. The ameliorative agent Vit-C recovered AST activity in pesticidal intoxicated embryos. AST activity in Group IV embryos remained more or less similar with that of the Gr III embryos indicating combination of Vit-C and atropinedid not further improved AST activity.

Alanine Aminotransferase (ALT) in Liver

The activity of ALT in liver is presented in Table 3 and Fig. 3. It is observed from the table that the activities of ALT in Gr I (control) increased gradually and reached its highest value in 14^{th} days of incubation. The values thus observed as1.16 IU/L and 1.88 IU/L in 8^{th} and 14^{th} days embryos respectively.

In Gr II, the ALT activity increased from its initial value of 8^{th} day to 14^{th} day embryo in dimecron treated groups. Thus the 8^{th} day embryos exhibited the lowest ALT activity of 2.11 I.U/L whereas in 14^{th} day, ALT activity was noted as 2.26 U/L due to pesticide treatment. The activity of ALT was increased due to the treatment of the pesticide. The pesticide treated group increase in ALT activity over the control and difference between the control and the experiment group is significant ($p<0.05$). Vit-C as ameliorative agent when injected gave a value nearer to control (Gr I) value. It is observed that Vit-C recovered ALT activity in pesticidal intoxicated embryos. To show the activity of atropine, the embryos were subjected to the treatment of Vit-C and atropine. The values thus showed towards activities like Gr III indicating a similar activity with that of the Vit-C treatment.

Conclusion

The organophosphate insecticide, dimecron when introduced into the fertilized hen's egg at a certain dose before incubation to see the action of the pesticide in developing chick embryo, shows a characteristic and interesting feature which has been studied and discussed in details.

Table 1. Effect of Vit-Cand atropine on brain acetylcholinesterase activity (nmol substrate hydrolysed/ min/ mg protein) in dimecron intoxicated chick embryos in different days of development.

Day	Control Gr I	Dimecron treatment Gr II	Dimecron + Vit-C Gr III	Dimecron + Vit-C + atropine Gr IV
4th	2.59 ± 0.04	1.41 ± 0.03*	2.26 ± 0.04[a]	2.00 ± 0.04 [a]
8th	10.86 ± 0.10	8.51 ± 0.11**	9.12 ± 0.10 [a]	8.68 ± 0.11 [a]
14th	14.42 ± 0.12	10.09 ± 0.11**	12.46 ± 0.11[a]	12.11 ± 0.11 [a]
Values are expressed as Mean ± SE (n= 5)				
Significant difference are indicated by *p< 0.05, **p< 0.01 when compared with control group of animals and [a]p< 0.05 when compared with pesticide treated and recovery group of animals; NS = not significant.				

Table 2. Effect of Vit-C and atropine on AST activity (IU/l) in dimecron intoxicated chick embryo in different days of development.

Day	Control Gr I	Dimecron treatment Gr II	Dimecron + Vit-C Gr III	Dimecron + Vit-C + atropine Gr IV
8th	1.64 ± 0.02	2.82 ± 0.04*	1.83 ± 0.02[a]	1.92 ± 0.01 [a]
14th	2.17 ± 0.01	3.56 ± 0.02*	2.34 ± 0.01 [a]	2.49 ± 0.01 [a]
Values are expressed as Mean ± SE (n= 5)				
Significant difference are indicated by *p< 0.05 when compared with control group of animals and [a]p< 0.05 when compared with pesticide treated and recovery group of animals.				

Table 3. Effect of Vit-C and atropine on ALT activity (IU/l) in dimecron intoxicated chick embryo in different days of development.

Day	Control Gr I	Dimecron treatment Gr II	Dimecron + Vit-C Gr III	Dimecron + Vit-C + atropine Gr IV
8th	1.16 ± 0.02	2.11 ± 0.02*	1.22 ± 0.01 [a]	1.29 ± 0.03 [a]
14th	1.88 ± 0.02	2.76 ± 0.02*	2.08 ± 0.02 [a]	2.22 ± 0.02 [a]
Values are expressed as Mean ± SE (n= 5)				
Significant difference are indicated by *p< 0.05 when compared with control group of animals and [a]p< 0.05 compared with pesticide treated and recovery group of animals.				

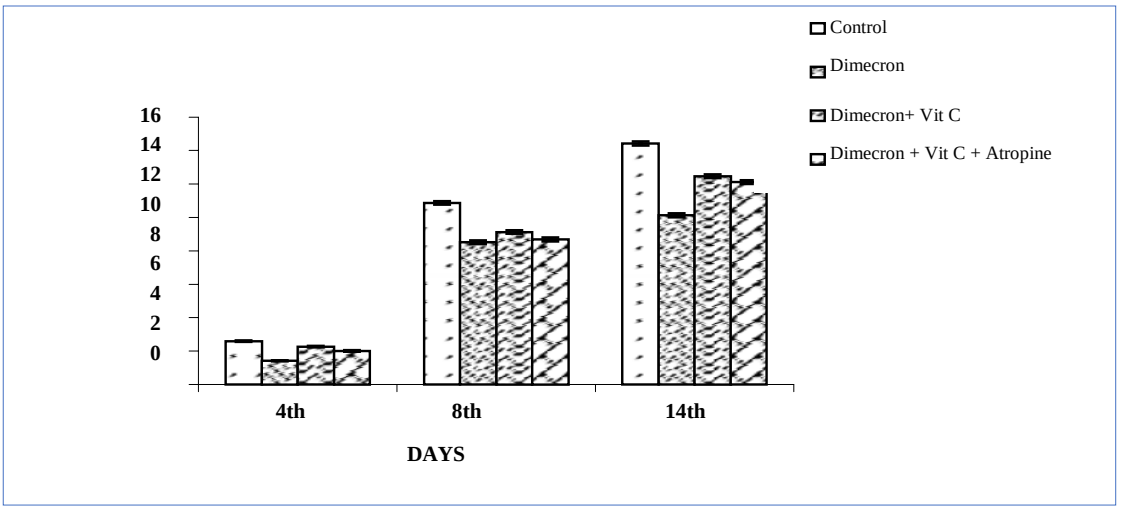

Fig.1. Effect of Vit-C and atropine on brain acetylcholinesterase activity (nmol substrate hydrolysed/ min/ mg protein) in dimecron intoxicated chick embryos in different days of development.

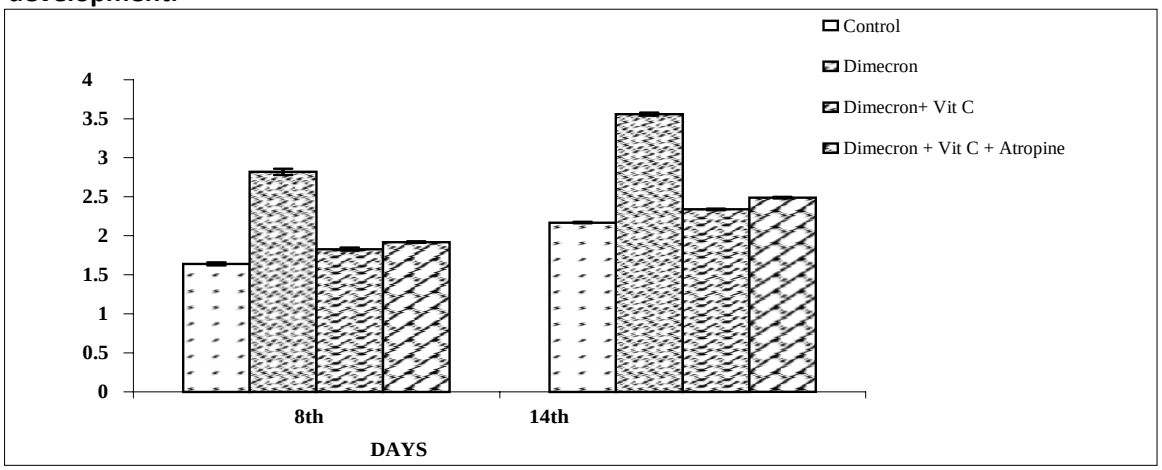

Fig.2. Effect of Vit-C and atropine on AST activity (IU/l) in dimecron intoxicated chick embryo in different days of development.

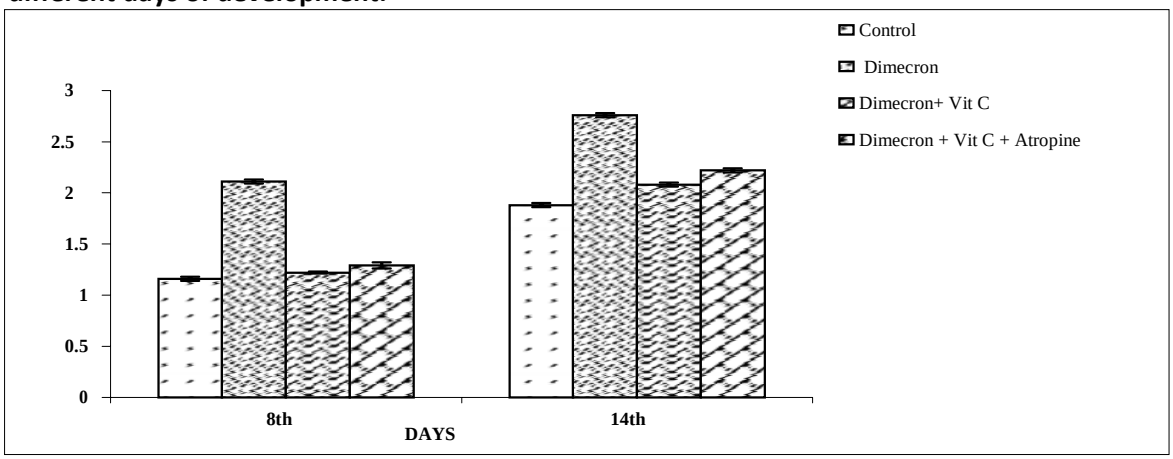

Fig. 3. Effect of Vit-C and atropine on ALT activity (IU/l) in dimecron intoxicated chick embryo in different days of development.

The study of acetylcholinesterase activity of brain which has been inhibited by the action of Dimecron. The over stimulation of cholinergic neuron initiates a process leading to structural brain damage of the organism, causing inhibition of the enzyme. The study of transaminase activity is very interesting as it shows both activities increased and that might have some correlation with the cellular damage and or stress condition. The vitamin used in the present study for amelioration of pesticide toxicity act as biological antioxidants. Vit C, that is considered as a well known antioxidant in biological system, has a power of reducing a variety of oxidative compounds especially free radicals. When ascorbate scavenge free radicals, the resultant dehydroascorbate is reduced to ascorbate to be used repeatedly.

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Conflict of interest:

Authors declare that there is no conflict of interest.

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