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Ameliorating effects of Vit-C in the activities of phosphatase enzymes in Dimecron intoxicated developing chick embryos

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ARTICLE DETAILS

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ABSTRACT

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 in different developmental stages. Present study shows the ameliorating effect of Vit-C in different days of study schedule.

INTRODUCTION

Fertilized avian egg is truly, a highly organized system containing all essential food and reserves for its normal development. This is interesting and peculiar since the avian embryo develops outside the mother's body and is provided with most elaborate protective mechanisms that really protects the embryo from various hazards. During development, as usual, the inert substances of the egg are converted into living tissues of the chick through external heat and atmospheric oxygen, with the exclusion of carbon dioxide as metabolic byproduct. This transformation is linked with a series of definite chemical events (**Romanoff, 1967**) and numerous changes occur in order of time. The pesticide residue also directly affects the embryos, disturbing the normal development and causing pathophysiological and morphological changes **(Varnagy, 1992)**

Organophosphorus pesticides are associated with toxic response and toxicity is reflected by change in metabolic enzymes. **Abou-Donia (1978a)** showed increase of acid phosphatase activity in hens plasma due to the effect of leptophos. Extensive report on phosphatase activities in the liver of several freshwater fishes was available (**Goel, 1975; Sastry and Agarwal, 1975; Dalela *et al*., 1976, 1978**). Generally, alkaline phosphatase activity in the liver and in the muscle were increased at lethal concentration and decreased at sublethal concentrations in the fish, *Channa* sp. (**Verma *et al*., 1982**) exposed to thiodon and rogor. **Shaikila *et al*. (1993)** reported the effect of carbamate pesticide, sevin, on the levels of both acid and alkaline phosphatase activities in liver and muscle of *Sarotheradon mossambicus* (Peters). Acid phosphatase level was increased particularly in the liver and there was a severe drop in tissue alkaline phosphatase enzyme activity. Significant increase in plasma acid phosphatase activity was noted by **Abou-Donia and Graham (1978b)** following long term low level administration of Leptophos to the comb of hens. Acid phosphatase is a hydrolytic enzyme and acts as a good indicator of stress condition in biological system (**Gupta *et al*., 1975; Verma *et al*., 1980**). **Dalela *et al*. (1980**) discussed about both liver acid and alkaline phosphatase activities in relation to the physiological stress induced by sublethal concentrations of phenol and pentachlorophenol in *Notopterus notopterus*. Maximum inhibition of acid phosphatase activity was noted by **Dalela *et al*. (1981**) in different tissues of *Notopterus notopterus* exposed chronically to phenolic compounds. However, **Koundinya and Ramamurthi (1982)** showed an increase of alkaline phosphatase activity in all tissues of freshwater teleost, *Sarotheradon mossambicus* following exposure to Sumithion.

MATERIALS AND METHOD

Fertilized eggs of Rhode Island Reds *(Gallus gallus*) were obtained from the Government poultry farm. Eggs were incubated at 37^0C ± 0.5^0C with an average relative humidity of 75% in the incubator for varying periods of time to obtain embryos at different epigenetic period. The organophosphate insecticide, 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), NADH, NADPH, EDTA and Vit-C (L-ascorbic acid) were purchased from Sisco Research Laboratory, 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 and kidney was done on 8^{th} and 14^{th} day embryos. A definite amount of liver or kidney was taken and homogenized in 0.9% sodium chloride. The homogenate was centrifuged at 4000 × g for 10 minutes at 4^0C. The supernatant was quantitatively collected and was used for further analysis. Both Acid phosphatase and alkaline phosphatase enzyme activity was determined by the method of **Walter and Schutt (1974)**. For statistical analysis analysis, student's t-test was used.

RESULT:

The amount of p-nitrophenol liberated by acid and alkaline phosphatases at 37^OC and pH 4.8 and pH 10.0 for acid and alkaline phosphatases in developing chick embryos are not same. The activities of both enzymes at constant temperature and different pH from liver and kidney as evidenced by the liberation of p-nitrophenol are recorded in tables and figures. It may be observed from the table that the activities of the enzymes vary during the study period.

Acid Phosphatases Activity (ACP) in Liver

The activity of acid phosphatase in liver is presented in **Table 1** and **Fig.**1**.**

Table 1: Effect of Vit-C and atropine on liver acid phosphatase activity (nmol/mg protein/hour) 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
8^{th}	1158.68 ± 52.38	1903.71 ± 59.71**	1369.33 ± 55.74[a]	1433.40 ± 55.02[b]
14^{th}	1672.21 ± 58.19	2263.83 ± 61.20**	1878.39 ± 60.85[b]	1941.60 ± 58.43[b]

Values are expressed as Mean ± SE (n= 5)

Significant difference are indicated by **p< 0.01 when compared with control group of animals and [a]p< 0.05 and [b]p< 0.01 when compared with pesticide treated and recovery group of animals

In control group, the specific activity of acid phosphatase enzyme in liver increases from its original condition of 8^{th} day as 1158.68 nmol/ mg of protein/hr to 1672.21 nmol/ mg of protein/hr in 14^{th} day. But due to the exposure of pesticide the acid phosphatase activity in liver has been increased from 8^{th} day to 14^{th} day of incubation. In terms of activities mention in the control the acid phospahatase activity shows an increased value and the difference between the control and the experiment is statistically significant (p<0.01).

Acid Phosphatases Activity (ACP) in Kidney

The activity of acid phosphatase in kidney is presented in **Table 2** and **Fig.2**

Table 2: Effect of Vit-Cand atropine on kidney acid phosphatase activity (nmol/mg protein/hour) 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	1486.82 ± 52.46	2492.24 ± 65.80**	1731.55 ±51.83[b]	1830.87 ± 54.53[b]
14th	1728.94 ± 51.59	2458.52 ± 65.28**	1977.56 ± 60.69[b]	2051.90 ± 61.16

Values are expressed as Mean ± SE (n= 5)

Significant difference are indicated by **p< 0.01 when compared with control group of animals and [b]p< 0.01 when compared with pesticide treated and recovery group of animals

The specific activity of acid phosphatase in kidney in control group also increases from the study period between 8th and 14th days of development. Thus the activities as 1486.82 nmol/ mg of protein/hr on 8th day and 1728.94 nmol/ mg of protein/hr for 14th day is observed. Due to the exposure of pesticide the acid phosphatase activity in liver has been decreased from 8th day to 14th day of incubation but this difference of activities between the days is not significant (p>0.05). The enzyme activity is increased due to the exposure of the pesticide and that shows a significant difference (p<0.01) when tested statistically through 't'-test.

When Vit-C was used for recovery of acid phosphatase activities both in liver and in kidney in Dimecron intoxication, it is found that the increased activities of enzymes in group II has been decreased (p<0.01).In the group IV when the recovery agent and atropine are administered against the pesticide intoxicated embryos it is observed that atropine treated groups behaves like that of ameliorating agent treated groups i.e. the enzyme activities is decreased due to the treatment of atropine.

Alkaline Phosphatase Activity (ALKP) in Liver

The activity of alkaline phosphatase in liver is presented in **Table 3 and Fig. 3**

Table 3: Effect of Vit-C and atropine on liver alkaline phosphatase activity (nmol/mg protein/hour) 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	1748.15 ± 54.18	1148.88 ± 47.39**	1455.98 ± 50.21[b]	1410.94 ± 50.57[b]
14th	2404.03 ± 60.98	1726.54 ± 57.77**	2103.27 ± 48.75[b]	2039.22 ± 60.49[b]

Values are expressed as Mean ± SE (n= 5)

Significant difference are indicated by **p< 0.01 when compared with control group of animals and [a]p< 0.05 and [b]p< 0.01 when compared with pesticide treated and recovery group of animals

In control group, the alkaline phosphatase activity of in liver increases from the study period between 8th and 14th days of development. Thus 8th day embryo shows aspecific activity of 1748.15 nmol/ mg of protein/hr whereas14th day embryo shows the specific activity as 2404.03 nmol/ mg of protein/hr. But due to the exposure of pesticide the alkaline phosphatase activity in liver has been increased from 8th day to 14th day of incubation when day-wise data is considered and this difference of activities between the days is significant (p<0.05). In terms of activities mention in the control, the alkaline phospahatase activity shows an decreased value and the difference between the control and the experiment is statistically significant (p<0.01).

Alkaline Phosphatase Activity (ALKP) in Kidney

The activity of alkaline phosphatase in kidney is presented in **Table 4 and Fig. 4**

The specific activity alkaline phosphatase of in kidney in control group, also increases from the study period between 8th and 14th days of development. Thus the activities as 2614.77 nmol/ mg of protein/hr on 8th day and 2876.64 nmol/ mg of protein/hr for 14th day are observed.

Table 4: Effect of Vit-Cand atropine on kidney alkaline phosphatase activity (nmol/mg protein/hour) 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	2614.77 ± 71.74	1886.42 ± 61.34**	2299.17 ± 62.32 [b]	2240.69 ± 61.46 [b]
14th	2876.64 ± 71.34	2169.74 ± 61.29**	2605.64 ± 65.40 [b]	2552.92 ± 63.34 [b]

Values are expressed as Mean ± SE (n= 5)

Significant difference are indicated by **p< 0.01 when compared with control group of animals and [b]p< 0.01 when compared with pesticide treated and recovery group of animals

But due to the exposure of pesticide the alkaline phosphatase activity in kidney has been decreased from 8th day to 14th day of incubation and there is a significance difference between control and treated embryos. The enzyme activity is decreased due to the exposure of the pesticide on both days (p<0.01).When Vit-C was used for the recovery of alkaline phosphatase activities from both liver and kidney against dimecron intoxication, it is found that the decreased activities of enzymes increased mostly due to the ameliorating agents. The ameliorative agent Vit-C recovered alkaline phosphatase activity in pesticidal intoxicated embryos. In the group IV when the recovery agent and atropine are administered against the pesticide intoxicated embryos it is observed that atropine treated groups also behaves like that of ameliorating agent treated groups thus showing the enzyme activities as increased due to the treatment of atropine.

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. Further, the toxicity develops due to the introduction of the pesticide is recovered by the use of ameliorating agent like Vit C that helps in ameliorating the toxicity symptoms. The present study shows that the activity of enzymes in terms of both acid and alkaline phosphatases, there is increase of acid phospahtasse but decrease in alkaline phossphatase measured in the liver as well as in kidney. The rise in acid phosphatase activity indicates a possible *in vivo* labilization of lysosomal membranes with the release of acid phosphatases. The decrease in alkaline phosphatase is due to the strong toxic effect of the pesticide accompanying the decrease in total protein as mentioned earlier. Thus it is concluded that the ameliorating agents Vit-C decreases the toxicity generated by dimecron pesticide in developing chick embryos. Further study related to reducing oxidative stress due to pesticide intoxication is to be done.

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FIGURES

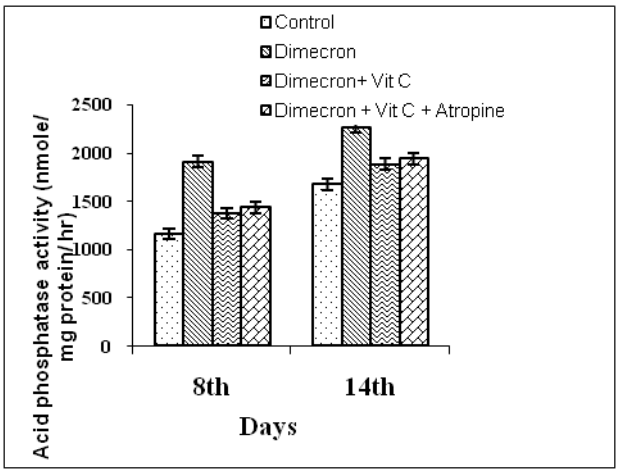

Fig. 1: Effect of Vit-C and atropine on liver acid phosphatase activity (nmol/mg protein/hour) in dimecron intoxicated chick embryo in different days of development

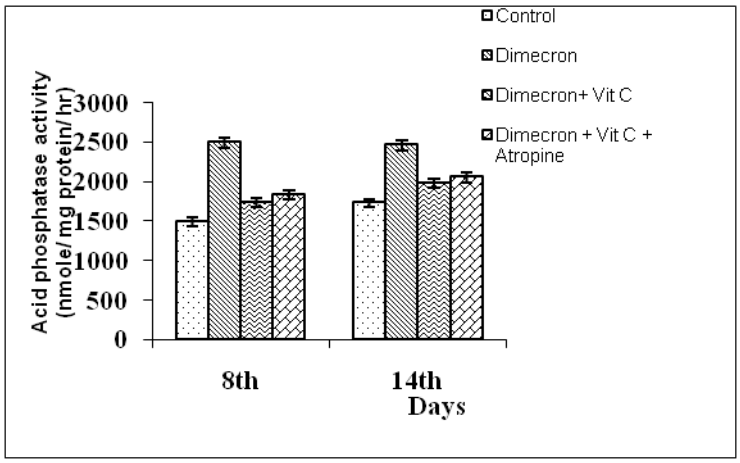

Fig. 2: Effect of Vit-C and atropine on kidney acid phosphatase activity (nmol/mg protein/hour) in dimecron intoxicated chick embryo in different days of development

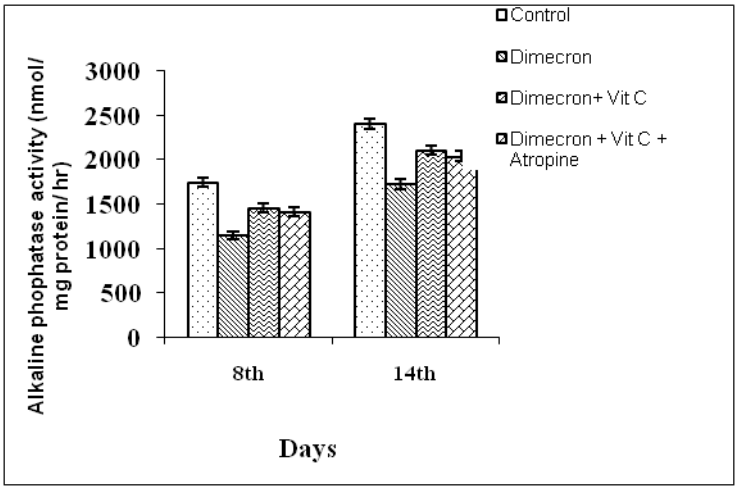

Fig. 3: Effect of Vit-C and atropine on liver alkaline phosphatase activity (nmol/mg protein/hour) in dimecron intoxicated chick embryo in different days of development

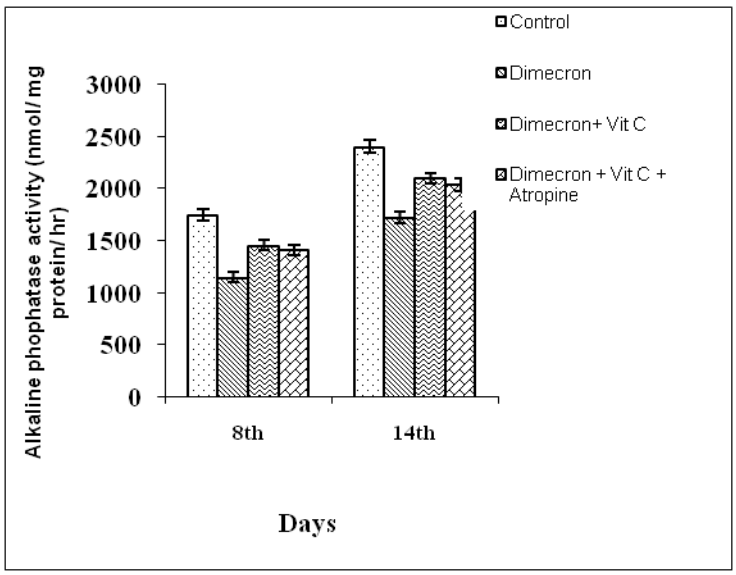

Fig. 4: Effect of Vit-Cand atropine on kidney alkaline phosphatase activity (nmol/mg protein/hour) in dimecron intoxicated chick embryo in different days of development