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ORIGINAL ARTICLE

Vitamin C as ameliorating agent in Phosphamidon treated chick embryos: A hematological study

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Pesticides used in agriculture and in public health to control insects, weeds and different vectors of diseases may also have adverse effects on the non-target living organisms. To delineate the toxicological profile on the broad spectrum organophosphate pesticide, Phosphamidon (Dimecron), the blood parameter has been analyzed in chick embryo in different period of epigenesis. The fertilized eggs were injected with 35 µg of dimecron in reference to control embryos which were injected with 0.9% normal saline solution. The ameliorating effect using vitamin C has also been considered in the present study. For hematological study, embryos were considered on 4^{th}, 8^{th} and 14^{th} days of incubation to collect blood samples. Parameters like hemoglobin concentration (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), were analyzed. The hematological parameters showed reduction in number of erythrocytes, Hb level, PCV, MCH and MCHC. Increase in MCV suggests an intensified compensating activity of hematopoeitic system in response to the hemolytic action of the pesticide. The change in the blood picture due to the action of the pesticide and the protective role of ameliorative agent has been discussed.

Keywords: Organophosphate, Phosphamidon, Chick embryo, Blood parameter.

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. Benke and Murphy (1975) reported organophosphorous compounds elicit considerable changes in the blood morphology of the animals. Szubartowska and Gromysz-Kalkowska (1992) described blood morphology in quails after poisoning with fenitrothion and showed that the pesticide reduced the number of erythrocytes, haemoglobin level and haematocrit value, but increased erythroblast and reticulocyte number. Gromysz-Kalkowska and Szubertowska (1986) reported the changes in blood of Pharaoh quails after Ekatin intoxication and also examined possible sex differences and the duration of disturbances in the birds towards intoxication. Carbaryl, however, caused changes in the abundance of various kinds of blood cells, when pigeons were exposed to the pesticide (Cox, 1993). Significant decrease of RBC number, haematocrit and haemoglobin levels were observed due to the injection of phenylhydrazine (PHZ) (Dornfest et al., 1983). Stino and Washburn (1970) reported that severe haemolytic anemia occurred in six week old chickens from a single injection of PHZ @ 30 mg/kg body weight. Furadan SP50 influenced in alterations of the blood parameters in blue-rock pigeon (Sharma and Saxena, 1998).

In view of the harmful effects caused by the ever increasing use of pesticides in our daily life, efforts were made to find out the agents capable of minimizing their toxicity. A number of ameliorating agents or bio-agents have been identified among naturally occurring and/or synthetic compounds (Kato, 1984). It is therefore desirable to study the effect of the vitamin C in the present study to recover the pesticidal effect as it is an important water soluble antioxidant is reported to neutralize reactive oxygen metabolites (ROMs) and reduce oxidative DNA damage (Frei, 1994).

Materials and Methods

Fertilized eggs of Rhode Island Reds *(Gallus gallus*) were obtained from the Government poultry farm. Eggs were incubated at 37°C ± 0.5°C 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. Vit-C (L-ascorbic acid) was purchased from Sisco Research Laboratory, India.

Before putting the fertilized eggs in the incubator, the eggs were washed with rectified spirit in order to avoid any contamination. 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). For Vit-C, 40 µg dose was selected for the recovery effect of the pesticide.

Study schedule

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

Group-I Control; Group-II Dimecron treated; Group-IIIA Dimecron+Vit-C

For hematological study about 3 ml of blood samples was collected from vitelline and allantoic blood vessels into a tube and eggs were sacrificed only on 4^{th}, 8^{th} and 14th days of incubation. Hematological parameters such as Total erythrocytes count (TEC). Haemoglobin concentration (Hb). Packed cell volume (PCV). Mean corpuscular volume (MCV). Mean corpuscular haemoglobin (MCH) and Mean corpuscular haemoglobin concentration (MCHC) were considered for the analysis of blood sample of embryo in different days of incubation. Total Erythrocyte Count (TEC) was done using hemocytometer with Neubauer's glass slide. Percentage of Haemoglobin was estimated by the alkali haematin method of Wu (1922). Packed Cell Volume (PCV) was estimated by an instrument called haematocrit. MCV was determined by dividing PCV in 1000 ml of blood and RBC/mm^3 of blood. It was expressed in cubic micron (μ^3). Mean corpuscular haemoglobin was determined by dividing haemoglobin in gms/1000 ml. of blood by RBC number/mm^3. MCH is expressed in micromicrograms ($\gamma\gamma$ or gamma gamma). Mean corpuscular haemoglobin concentration was determined by dividing haemoglobin in grams/100 ml of blood by the volume of packed red cell/1000 ml of blood multiplied by 100. This is expressed as percentage of haemoglobin of the total weight of an erythrocyte.

Statistical analysis

For statistical analysis, the data were analyzed with Student's 't' test (Fisher 1963).

Results and Discussion

Total count of red blood cells of different days of incubation has been presented in Table 1. Variations are observed both in control as well as in the treated embryos. The number of red blood cell s appear to be less than that of the control in all the days of study and the number becomes almost reduced to half on all the days. In group III embryos where dimecron and Vit-C were administered, the number of RBC increased ($p<0.05$) from group II embryos (dimecron treated embryos) on 4^{th} days. Similar increase is also observed on 8^{th} and 14^{th} day incubated embryos.

Table 1. Effect of Vit-C on total erythrocyte count in million/cu mm of blood in dimecron intoxicated chick embryos in different days of development.

Day	Control	Dimecron treatment	Dimecron+Vit-C
	Gr I	**Gr II**	**Gr IIIA**
4th	1.51 ± 0.10	0.86 ± 0.05*	1.31 ± 0.11[a]
8th	2.15 ± 0.11	1.10 ± 0.08**	2.10 ± 0.08[a]
14th	3.06 ± 0.13	2.15 ± 0.06**	3.59 ± 0.12[a]

Values are expressed as Mean ± SE; Significant difference are indicated by *p<0.05 and **p<0.01 when compared with control group of animals and [a]p<0.051when compared with pesticide treated and recovery group of animals.

The hemoglobin concentration which is expressed in gm/100 ml blood exhibits variation throughout the period under study and has been represented in Table 2. Maximum percentage of Hb is reduced in initial stage of the development which is somehow recovered on 8th day but still the pesticidal stress persists on 14th day. In group-III embryos the percentage of Hb recovered as 2.96, 3.20 and 5.10 gm/100 ml of blood for 4, 8 and 14th days of incubation respectively when Vit-C was administered on dimecron intoxicated embryos.

Table 2. Effect of Vit-C on percentage of hemoglobin in mg/100 ml of blood in dimecron intoxicated chick embryos in different days of development.

Day	Control	Dimecron treatment	Dimecron+Vit-C
	Gr I	**Gr II**	**Gr IIIA**
4th	3.38 ± 0.09	1.48 ± 0.06**	2.96 ± 0.07[b]
8th	3.41 ± 0.05	1.62 ± 0.07**	3.20 ± 0.08[b]
14th	5.31 ± 0.11	2.43 ± 0.06**	5.16 ± 0.10[c]

Values are expressed as Mean ± SE;Significant difference are indicated by **p<0.01 when compared with control group of animals and [a]p<0.05, [b]p<0.01 and [c]p<0.001when compared with pesticide treated and recovery group of animals.

Packed cell volume does not remain constant, but exhibits variation throughout the period under study and has been shown in Table 3. In Group-III embryos the PCV recovered as 4.02, 6.22 and 13.96 for 4, 8 and 14th days of incubation respectively when Vit-C was administered on dimecron intoxicated embryos.

Table 3. Effect of Vit-C on packed cell volume of blood in dimecron intoxicated chick embryos in different days of development.

Day	Control	Dimecron treatment	Dimecron+Vit-C
	Gr I	**Gr II**	**Gr IIIA**
4th	4.36 ± 0.08	4.40 ± 0.06[NS]	4.02 ± 0.05[NS]
8th	6.84 ± 0.11	4.68 ± 0.07[*]	6.22 ± 0.10[b]
14th	14.22 ± 0.22	10.42 ± 0.16[**]	13.96 ± 0.13[b]

Values are expressed as Mean ± SE; Significant difference are indicated by *p< 0.05 and **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; NS = not significant.

The MCV expressed in μ^3 exhibits variation both in control and in treated series of all age group considered. The values have been presented in Table 4. The increase in MCV in comparison to control group is statistically significant (P<0.05). The Vit-C administration against dimecron intoxicated embryo recovered MCV value of 106%, 96% and 100% of 4th, 8th and 14 days.

Table 4. Effect of Vit-C on mean corpuscular volume (MCV) in μ^3 of blood in dimecron intoxicated chick embryo in different days of development.

Day	Control Gr I	Dimecron treatment Gr II	Dimecron+Vit-C Gr IIIA
4th	28.87 ± 4.20	51.16 ± 11.29*	30.68 ± 3.19[a]
8th	31.81 ± 3.2	42.54 ± 3.14*	34.64 ± 2.14[a]
14th	38.85 ± 3.17	48.36 ± 2.14*	38.88 ± 2.11[a]

Values are expressed as Mean ± SE;Significant difference are indicated by *p<0.05 when compared with control group of animals and [a]p<0.05when compared with pesticide treated and recovery group of animals.

The MCH expressed in $\gamma\gamma$ exhibits variation both in control and in treated series of all age group considered. The values have been presented in Table 5. The MCH also changes due to the exposure of pesticide. The decrease in MCH in comparison to control group is statistically significant (p<0.05 and p<0.01). In group III embryos treated with Vit-C against dimecron intoxication, shows a value of 22. 26 $\gamma\gamma$ on 4th day. However MCH value became 15.00 and 14.22 $\gamma\gamma$ on 8th and 14th day embryos.

Table 5. Effect of Vit-C on mean corpuscular haemoglobin (MCH) in $\gamma\gamma$/cell in dimecron intoxicated chick embryo in different days of development.

Day	Control Gr I	Dimecron treatment Gr II	Dimecron+Vit-C Gr IIIA
4th	22.38 ± 0.52	17.28 ± 0.33**	22.26 ± 0.16[a]
8th	15.88 ± 0.22	14.72 ± 0.35*	15.00 ± 0.26[NS]
14th	14.52 ± 1.11	11.34 ± 0.60*	14.22 ± 0.33 [b]

Values are expressed as Mean ± SE;Significant difference are indicated by *p<0.05 and **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; NS=not significant.

The MCHC exhibits variation both in control and in treated series of all age group considered. The values have been presented in Table 6. The decrease in MCH in comparison to control group is statistically significant (P<0.001). In group III embryos treated with Vit-C against dimecron intoxication, shows a value of 73.63 on 4th day. However MCHC value became 49.67 and 36.53 on 8th and 14th day embryos.

Table 6. Effect of Vit-C on mean corpuscular haemoglobin concentration (MCHC) in dimecron intoxicated chick embryo in different days of development.

Day	Control Gr I	Dimecron treatment Gr II	Dimecron+Vit-C Gr IIIA
4th	77.52 ± 0.38	33.63 ± 0.17***	73.63 ± 0.24[c]
8th	49.85 ± 0.17	34.61 ± 0.17***	49.67 ± 0.29[b]
14th	37.34 ± 0.16	23.32 ± 0.13***	36.53 ± 0.17[b]

(Values are expressed as Mean ± SE; Significant difference are indicated by ***p<0.001 when compared with control group of animals and [a]p<0.05, [b]p<0.01 and [c]p<0.001 when compared with pesticide treated and recovery group of animals).

Analysis of the present hematological study justify the statement that the direction and intensity of hematological changes in developing chick embryos intoxicated with dimecron exhibit some degree of relationship among the stages studied in the embryo. Considerable reduction in the number of erythrocyte, Hb%, changes of packed cell volume (PCV) agree with the results studied by large number of authors using OP compound. (Ali and Shakon, 1981; Gromysz-Kalkowska et al, 1981; Gromysz-Kalkowska and Szubertowska, 1986; Szubartowska and Gromysz-Kalkowska, 1992; Al-Qarawi et al., 1999; Khalaf-Allah, 1999). This reduction in the

number of erythrocytes in dimecron intoxication may be explained as a result of hemorrhage and clotting. Extensive hemorrhage to the pulmonary parenchyma or veiling clots in the arterioles due to pesticide intoxication and the hemolytic action of the pesticide (Gromysz-Kalkowska et al, 1981) also deserve mentioned. The present study of an increase in mean corpuscular volume in the specified dose suggests an intensified compensating activity of the hematopoetic system in response to hemolytic action of Dimecron. The present results are in consistent with the study of (Gromysz-Kalkowska et al, 1981) on quails intoxicated with OP pesticide. Again the fall of erythrocyte count and hemoglobin level during different developmental periods due to dimecron intoxication with a simultaneous tendency to fall in the hematocrit value can be related with the intensive decrease of reticulocyte count at this time. In the present study, the MCH and MCHC decrease is presumed to be decrease rate of reticulocyte which contributed to non-alleviation of MCH and MCHC. When Vit-C as ameliorating agent has been administered to the developing chick embryo against Dimecron intoxication, it may be stated that ascorbic acid in the present study increases the antioxidant effectiveness by acting aqueous free radical trap.

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