International Journal of Experimental Research and Review (IJERR)

ISSN: 2455-4855 (Online) ***Original Article***
Received: 4th December, 2015; **Accepted:** 19th January, 2016; **Published:** 30th January, 2016

DOI: https://doi.org/10.52756/ijerr.2016.v2.005

Ameliorating effects of Vit-C on protein and nucleic acid content in dimecron intoxicated chick embryos

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Abstract

Dimecron when introduced into the fertilized hen's egg at a certain dose before incubation shows a characteristic and interesting feature which has been studied and discussed. A quantitative study of proteins from different organs viz. liver, kidney and brain has been made in the present study. A significant reduction in the quantity of the protein of all the organs is observed. The study witnessed the activity of DNA and RNA that undergoes a decline in its quantity by the action of the pesticide. The toxic effect of pesticides is recovered by the use of ameliorating agent like Vit C.

Keywords: Atropine, DNA, organophosphate, protein, RNA, Vit-C.

Introduction

Fertilized avian egg is a highly organized system containing all essential food and reserves for its normal development. 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.

Pesticides act either as a selective toxicant or may display rather broad spectrum adverse biological activities. Studies pertaining to the effects of pesticides on protein content and the level of nucleic acid have been recorded. Jaroli and Sharma (2005) reported the effect of chlorpyrifos on the liver of *Channa* and exhibited notable alteration. A marked change in the nucleic acid levels and protein contents has been observed in DDT and dieldrin exposed rats (Bhatia et al., 1973). A significant decrease in hepatic protein valuehas been reported in long term exposure to dieldrin in catfish (Bano, 1982; Singh and Srivastav, 1995). Fenvalerate induces the reduction in protein level in catfish (Tripathi and Verma, 2004). Tripathi et al., (2003) reported the dimethoate intoxication decreases the total protein and nucleic acids level in *Channa punctatus*. In a study, with sublethal levels of DDT, malathion on liver and muscle proteins of *Sarotherodon mossambicus* (Peters) Ramalingam and Ramalingam (1982) reported a decline in levels of protein indicating the role of toxicant in maintenance of energy supply. Ahmed et al., (1986) reported the decrease in total protein content of rat brain due to exposure of dieldrin at different time

intervals. Ansari et al., (1988) studied diazinon toxicity in Zebra fish exposed for 24 to 168 hours and showed a significant time dependent effect on the nucleic acid and protein content. However, the results of the treatment of diazinon - an organophosphate insecticide on nucleotide and amino acid contents of chick embryos showed a decrease value (Kitoset et al., 1981; Kushaba-Rugaaju and Kitos, 1985). Very few studies have been done in response to qualitative changes of serum protein due to the exposure of pesticide. Anees (1974), however, reported a change in serum protein of a freshwater teleost, *Channa punctatus* (Bloch) exposed to organophosphate insecticides.

Methodology

Fertilized eggs of Rhode Island Reds *(Gallus gallus*) were obtained from the Government poultry farm, Haringhata. Eggs were incubated at 37^0C $\pm$ 0.5^0C with an average relative humidity of 75% in the incubator. The organophosphate insecticide, Dimecron, was used for the present study. Technical grade Dimecron (85% SL purity) was available from Hindustan Ciba-Geigy Limited, India. All the research grade chemicals used in this study. Preincubated eggs were injected with desired doses of insecticide according to the method of McLaughlin et al., (1963). The pesticide 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

Estimation of Protein

A definite amount of tissue samples (liver, kidney and brain) was taken. For estimation of total quantity of protein present in different tissues, the method of Lowry et al., (1951) was followed.

Estimation of Deoxyribonucleic acid (DNA)

The DNA was estimated through diphenylamine reaction.

Estimation of Ribonucleic acid (RNA)

The RNA was estimated through orcinol reagent.

For statistical analysis, student's t-test was used.

Result

Study of Protein from liver

The quantity of liver protein has been shown in Table 1 and Fig. 1. It may be observed from the table that the concentration of protein in both the days varies. The quantity of protein, in control group, increases from 8^{th} day to 14^{th} day and maximum quantity is available as 27.93mg/100mg of liver tissue on the 14^{th} day. However, in pesticide treated group, the level of protein also increases from 8^{th} day to 14th day embryo but in comparison to control the protein quantity has been decreased in both the days. The concentration of protein in the liver appears to be less than that of control and when calculated by percentage value the 8^{th} embryo shows maximum reduction (21.00%) of protein than the 14^{th} day embryo. Statistical analysis shows a significant difference between control and treated groups ($p< 0.01$). The protein that has gone to a decreased condition due to dimecron has been recovered in group III embryos treated with Vit-C. The level of protein in group III is higher than pesticide treated groups indicating a recovery of 90.94% on 8^{th} day and 91.40% on 14^{th} day for ascorbic acid. In Group IV, the ameliorating agent and

atropine are given against the pesticide intoxicated embryos, it is found that the protein quantity so far available in Group IV is more or less similar with the quantity of protein available in Group III embryos.

Study of Protein from kidney

The quantity of protein estimated from the kidney has been presented in Table 2 and Fig. 2. In control group, the highest concentration is however is observed on 14^{th} day group of embryos. But in treatment group, the protein content reduced. The level of protein in the kidney appears to be less than that of the control value. When the amount of protein is calculated as percentage, the 14^{th} day embryo shows maximum reduction (27.42 %) ($p<0.01$) of protein than the 8^{th} day embryo ($p<0.05$). The decreased condition of the protein due to Dimecron has been recovered in Group III embryos treated with Vit-C. The level of protein in group III is higher than pesticide treated groups indicating a recovery of 77.48% on 8^{th} day and 84.10% on 14^{th} day for ascorbic acid. In group IV, when the ameliorating agent and atropine are given against the pesticide intoxicated embryos, it is seen that the quantity of protein in Group IV is more closely associated with the quantity of protein available in Group III embryos.

Study of Protein from brain

The quantity of protein estimated from brain tissue has been shown in Table 3 and Fig. 3. The quantity of protein increases in control group gradually from 4^{th} day to 14^{th} day incubated embryos and reaches maximum on 14^{th} day of incubation. The lowest and the highest concentration observed in 4^{th} and 14^{th} days embryos respectively. The level of protein which initially shows a level in 4^{th} day embryo that declines on 8^{th} day embryo and rises again on 14^{th} day of incubation in treated Group II. In 't' test significant differences exists between control and treated groups. The decrease in concentration of protein between control and treated embryos is significant ($p<0.01$).In Group III, the decrease concentration of protein due to dimecron intoxication, has been recovered significantly ($p<0.05$) when treated with Vit-C. When the ameliorating agent and atropine are given against the pesticide intoxicated embryos, it is found that the protein quantity in brain so far available in Group IV shows similarity with the quantity of protein available in Group III embryos.

Study of nucleic acids

Deoxyribonucleic Acid (DNA)

The quantity of DNA estimated from fresh brain tissue has been shown in Table 4 and Fig. 4. In control Group, DNA concentration is highest in 4^{th} day embryo but the amount declines on 8^{th} day followed by a further decline on 14^{th} day. In Group II, the level of DNA in pesticide treated groups initially shows a highest level but the concentration falls down like the control and it shows a lowest level in 14^{th} days. It is observed that the percentage of decrease ($p<0.05$) in the level of DNA is highest on 8^{th} day. In Group III, the treatment with ascorbic acid resulted in the increase of DNA in comparison to the pesticide treated animal throughout the phases of development under study. When ascorbic acid is treated as a recovering agent, it shows a good recovery of DNA in different period of study. In Group IV, DNA concentration values remains towards the value of ascorbic acid treatment.

Ribonucleic Acid (RNA)

The concentration of fresh brain RNA has been documented in Table 5 and Fig. 5. It has been shown that the concentration of RNA varies in the selected period of study.

Table 1. Effect of Vit-C and atropine on Liver protein content (mg/ 100 mg) 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
8th	10.60 ± 0.14	8.37 ± 0.11** (21)	9.64 ± 0.16[a] [90.94]	9.31 ± 0.13 [a] [87.83]
14th	27.93 ± 0.26	23.36 ± 0.18**	25.53 ± 0.19[a] [91.40]	24.56 ± 0.19[a] [87.93]
Values are expressed as Mean ± SE (n= 5); Values in parentheses indicate % decrease in relation to control; Values in [ ] indicate recovery in relation to control; Significant difference are indicated by **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.				

Table 2. Effect of Vit-C and atropine on kidney protein content (mg/ 100 mg) 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
8th	5.95 ± 0.13	4.07* ± 0.13 (31.59)	4.61 ± 0.14[a] [77.48]	4.43 ± 0.16 [a] [74.45]
14th	20.64 ± 0.20	14.98** ± 0.22 (27.42)	17.36 ± 0.19 [a] [84.10]	17.12 ± 0.23 [a] [82.95]
Values are expressed as Mean ± SE (n= 5); Values in parentheses indicate % decrease in relation to control; Values in [ ] indicate recovery in relation to control; Significant difference are indicated by *p< 0.05 and, **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 3. Effect of Vit-C and atropine on brain protein content (mg/ 100 mg) 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	7.59 ± 0.12	5.14** ± 0.10 (32.27)	6.37 ± 0.12 [a] [83.92]	6.11 ± 0.11 [a] [80.5]
8th	8.93 ± 0.11	5.08** ± 0.11 (43.11)	6.91 ± 0.14 [a] [77.37]	5.41 ± 0.11 [a] [71.78]
14th	10.56 ± 0.12	7.70** ± 0.12 (27.08)	8.52 ± 0.13 [a] [79.16]	8.27 ± 0.14 [a] [78.31]
Values are expressed as Mean ± SE (n= 5); Values in parentheses indicate % decrease in relation to control; Values in [ ] indicate recovery in relation to control; Significant difference are indicated by **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.				

Table 4. Effect of Vit-C and atropine on deoxyribonucleic acid content (mg/ 100 mg) of brain tissue 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	0.615 ± 0.005	0.519* ± 0.007	0.585 ± 0.005 [a]	0.558 ± 0.005 [a]
8th	0.475 ± 0.004	0.315* ± 0.005	0.382 ± 0.003 [a]	0.371 ± 0.002 [a]
14th	0.369 ± 0.002	0.288* ± 0.004	0.328 ± 0.006 [a]	0.311 ± 0.005 [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; NS = not significant				

Table 5. Quantity of brain ribonucleic acid (mg/ 100mg) in control, Vit-C and atropine treated groups in dimecron intoxicated chick embryos at different period of development.

Day	Control Gr I	Dimecron treatment Gr II	Dimecron + Vit-C Gr III	Dimecron + Vit-C + atropine Gr IV
4th	0.392 ± 0.009	0.311 ± 0.007*	0.366 ± 0.004 [a]	0.352 ± 0.005 [a]
8th	0.574 ± 0.009	0.355 ± 0.004**	0.483 ± 0.006[a]	0.461 ± 0.004 [a]
14th	0.674 ± 0.006	0.511 ± 0.005**	0.591 ± 0.007 [a]	0.581 ± 0.006 [a]
Values are expressed as Mean ± SE (n= 5); 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.				

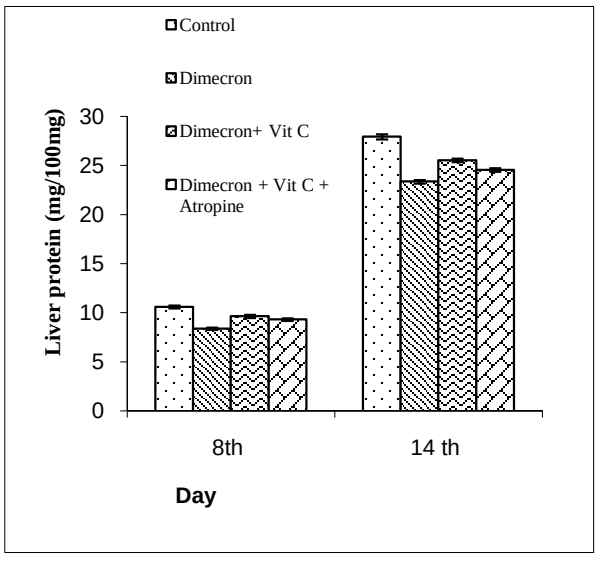

Fig.1. Effect of Vit-C and atropine on Liver protein content (mg/ 100 mg) in dimecron intoxicated chick embryos in different days of development.

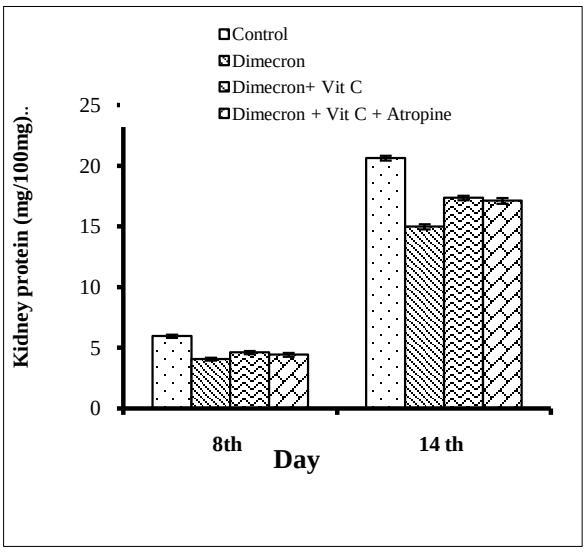

Fig.2. Effect of Vit-C and atropine on Kidney protein content (mg/ 100 mg) in dimecron intoxicated chick embryos in different days of development.

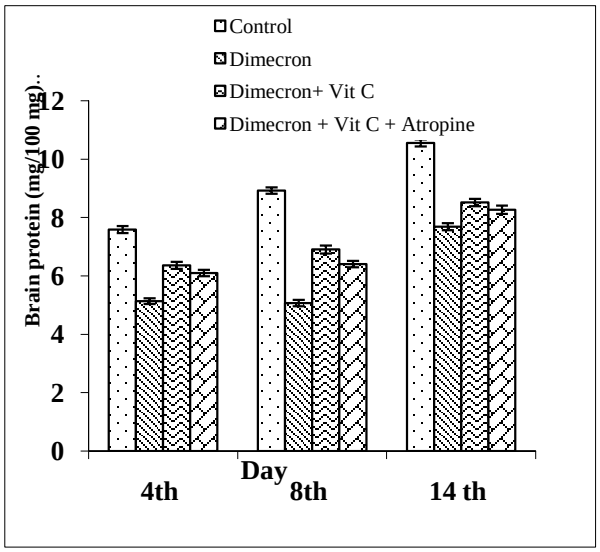

Fig. 3. Effect of Vit-C and atropine on brain protein content (mg/ 100 mg) in dimecron intoxicated chick embryos in different days of development.

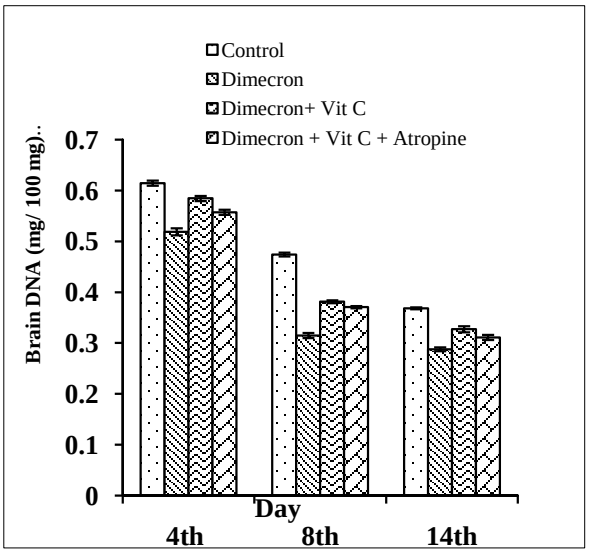

Fig. 4. Effect of Vit-C and atropine on deoxyribonucleic acid content (mg/ 100 mg) of brain tissue in dimecron intoxicated chick embryos in different days of development.

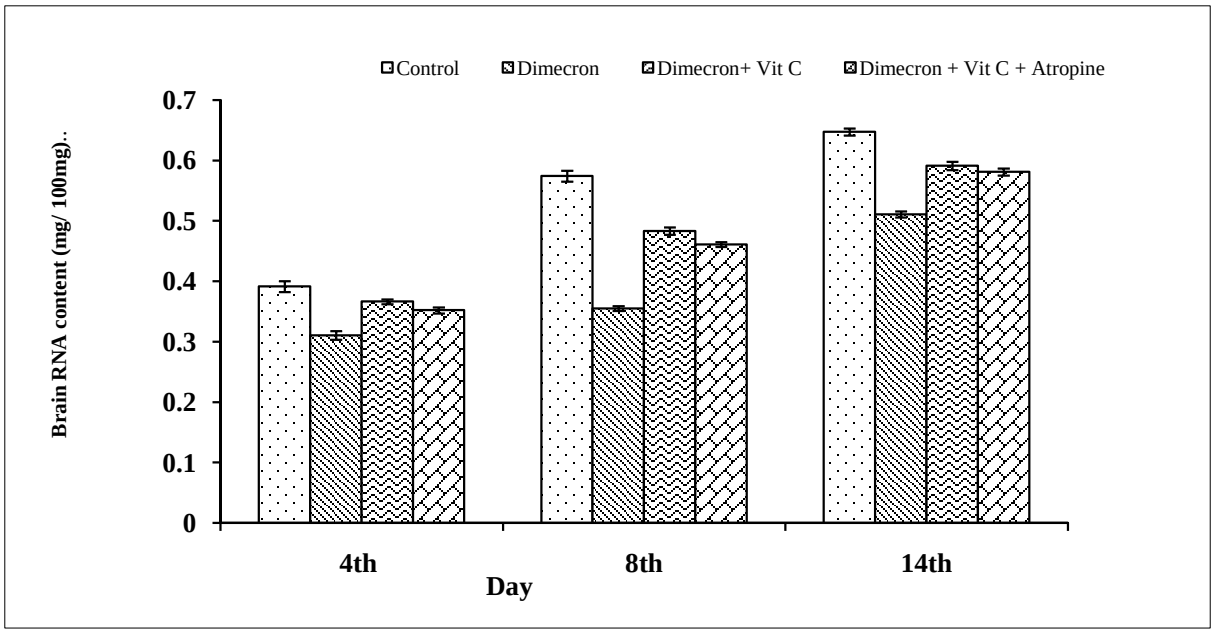

Fig. 5. Effect of Vit-C and atropine on ribonucleic acid content (mg/ 100 mg) of brain tissue in dimecron intoxicated chick embryos in different days of development.

In Control group, it has been shown that the concentration of RNA remains initially low but gradually increases on the following days and maximum amount of RNA is observed on14th days embryos. This increase in the level between days is statistically significant ($p<0.01$). Pesticide exposure has altered the concentration of RNA in the brain taken from the chick embryo in different days of development. The increased level of RNA between 4th and 8th day and also between 8th day and 14th day is statistically significant ($p<0.05$). Due to the treatment with Vit C (Group III), resulted in the increase of concentration of RNA in comparison to group II pesticide treated embryos. Thus maximum quantity of RNA observed on 14th day embryos in both the treatment. In Group IV, the atropine treatment however keeps the RNA concentration values towards the value of ascorbic acid treatment.

Conclusion

Dimecron when introduced into the fertilized hen's egg at a certain dose before incubation 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, helps in ameliorating the toxicity symptoms. It is well known that morphogenetic process is nothing but a chain of reactions in each step and each step of morphogenesis is controlled and determined by some biochemical trigger. Thus a quantitative study of proteins from different organs viz. liver, kidney and brain has been made in the present study. A significant reduction in the quantity of the protein of all the organs is observed. Further the study witnessed the activity of DNA and RNA that undergoes a decline in its quantity by the action of the pesticide. The results of the study can be attributed to the strong toxic effect of the pesticide. The vitamin which is 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 dehydro-ascorbate is reduced to ascorbate to be used repeatedly. The ameliorative agent has protective role in minimizing the effects in all the parameters studied. Thus it is concluded that the ameliorating agent increases the tolerance against the toxicity and decreases the toxicity generated by dimecron pesticide in developing chick embryos.

Acknowledgement

I am thankful to Professor C. R. Sahu, University of Kalyani for all the support during this study period.

Conflict of interest:

Author declare that there is no conflict of interest.

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