



AMELORATING POTENCY OF VITAMIN E ON HAEMATOLOGICAL PARAMETERS IN CADMIUM INDUCED TOXICITY IN LABORATORY CHICKS

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ABSTRACT

The present study was designed to check the ameliorating effect of vitamin E on haematological parameters in cadmium chloride induced toxicity in laboratory chicks (Gallus gallus domesticus). Developing chicks (100±20 gm body weight, 2-3 weeks old) were used as experimental animals. Chicks were randomly divided into three groups viz., A, B and C. Group A and B administered with cadmium chloride orally (5.0 mg/100 gm body weight) on each alternate day for 30 days. In addition to cadmium supplementation, group B also administered with vitamin E (0.5 IU/100 gm body weight) intramuscularly on each alternate day for 30 days. Chicks of Group C were considered as control and fed on basal diet and saline water only. Experiment was chronically continued for 30 days. After scheduled treatment chicks were sacrificed and blood collected through cardiac puncture for the experiments. Results showed significant reduction ($p < 0.05$) in blood parameters (RBC, WBC, Hb and PCV) were observed in chicks treated with cadmium chloride in group A. However, supplementation of vitamin E in cadmium chloride treated chicks (group B) resulted in marked improvements in haematological parameters. Present study indicate that vitamin E is a potent antioxidant that inhibit toxicity produced by cadmium chloride by chain breaking mechanism. The treatment of vitamin E normalized these haematological values up to the control level, signifying its protective effect in cadmium induced toxicity.

KEY WORDS

Cadmium chloride, Vitamin E, Haematological parameters, Anaemia, Chicks.

INTRODUCTION

Cadmium (Cd) can be considered the most toxic heavy metals [1]. Raised concentrations of Cd in soil may be found as a result of industrial activities (e.g. mining) or agricultural activities (e.g. sewage sludge, phosphate fertilizers, and pesticides) containing high concentrations of Cd [2]. Consumption of contaminated water is the major way by which humans are exposed to Cd [3] and the maximum allowable level in drinking-water is 0.005 mg/dL [4].

Animals can be exposed to Cd pollution by inhalation of polluted air, ingestion of polluted food and drinking of

polluted water [5,2,6]. It is absorbed from gastrointestinal tract to blood, and cadmium is taken up from the blood into the tissues. The Cd accumulates in human and animals tissues, especially the liver and kidney, causing their damage [2]. Cd also causes reduced feed intake and weight loss, decreased RBC (red blood cell) and Hb (blood haemoglobin) values [7, 8] and anaemia in Cd exposed animals.

Cadmium induced injury to liver and kidney organs has been attributed to its ability to enhance free radical formation in vivo [9]. It also induces various pathological changes in liver tissues including engorgement of blood

vessels, congestion, vacuolar degeneration of hepatocytes, necrosis of pancreatic cells and fatty changes in the pancreatic hepatocytes [10,11]. Chronic Cd toxicity caused an oxidative stress throughout lipid peroxidation and consumption of some antioxidant systems in broiler [12]. After absorption, this metal causes variety of toxic effects on various body tissues of both human and animals [13].

Cadmium and its compounds may be toxic in certain forms and concentrations. It is classified as a human carcinogen by the North Carolina national toxicology program. The toxic effects of cadmium on human health were first known in 1858 [14]. Cadmium accumulates in soft organs and results in toxicity [15]. Acute cadmium exposure produced toxicities to the lung, liver, testes and brain, while chronic exposure to cadmium often leads to renal dysfunction, anaemia, osteoporosis, and bone fractures [16]. In addition, Cd exposure also leads to carcinogenesis, cardiovascular dysfunction and *itai-itai* disease.

Cadmium has been demonstrated to stimulate free radical production, resulting in oxidative deterioration of lipids, proteins and DNA, and initiating various pathological conditions in humans and animals [17, 18, 19, 20]. Therefore, estimation of free radical generation and antioxidant defense has become an important aspect of investigation in mammals. Vitamin E (α -tocopherol) is naturally occurring antioxidants that play important role in animal health by inactivating harmful free radicals produced through normal cellular activity and from various stressors.

The antioxidant function of vitamin E could, at least in part, enhance immunity by maintaining the functional and structural integrity of important immune cells [21, 22, 23, 24]. Because the health problems induced by many environmental pollutants, much efforts have been expended in evaluating the relative antioxidant potency of vitamin E. Therefore, the present study deals with determining the efficiency of vitamin E in combating the toxicity of cadmium chloride (CdCl_2) on hematological parameters in laboratory chicks.

MATERIAL AND METHODS

Animals and treatment:

The experiment was carried out on newly hatched domestic chicks purchased from Uttarakhand Village Poultry Project (State Govt. Poultry Farm), Bin, Pithoragarh (Uttarakhand). These chicks were reared in

battery cages under laboratory conditions at existing room temperature and relative humidity for 2-3 weeks. They were fed on commercial food (Starter, Grower and Finisher) purchased from the local market (Godrej Company) and tap water *ad libitum*. Healthy male and female chicks (body weight 100 ± 20 gm) were used in present study. The selected chicks were randomly divided into 3 groups (A, B and C), each containing 6 chicks.

Group A:

Chicks were orally administered with sub lethal chronic dose of Cadmium chloride (5 mg/100 gm body weight) on each alternate day for 30 days.

Group B:

Chicks were treated with cadmium chloride (5 mg/100 gm body weight) and also administered with vitamin E intramuscularly (0.5 IU/100 gm body weight) on each alternate day for 30 days simultaneously.

Group C:

Chicks were administered commercial food with saline only used as control.

Source of chemicals:

Vitamin-E (α -tocopherol) and EDTA were purchased from Sigma Chemical Co. (St. Louis, Mo, USA). Cadmium chloride (CdCl_2) was procured from Glaxo (India).

Haematological study:

Blood samples were collected through cardiac puncture using 3ml disposable syringe and directly transferred into a labeled test tube containing anticoagulant (EDTA). The samples were kept in an ice box, using icepacks and transferred to the laboratory for measuring the haematological parameters (RBC, WBC, Hb and PCV). Blood cells were counted by Neubauer double haemocytometer using Haem's and Turek's solution as respective diluting fluids. Haemoglobin percentage was measured by cyanomethemoglobin method. PCV was determined by Wintrobe's haematocrit tube method. The protocol of the study was in conformity with the guidelines of the Institutional Ethical Committee constituted by Dept. of pharmaceutical Science, Kumaun University, Nainital, Uttarakhand and Ministry of Environment, Forests and Climate Change, Govt. of India.

Statistical Analysis:

Mean and standard error were calculated, and data were analyzed using standard methods. Parameters of all treatments were compared using Student's "t" test. Data were subjected to one-way ANOVA for calculating

the significance difference between the treatments. P-values less than 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

As presented in Table 01 haematological parameters RBC, WBC, Hb and PCV were decreased significantly ($p < 0.05$) in cadmium treated chicks as compared to control group. While, in chicks co-administered with

cadmium and vitamin E simultaneously, the value of these parameters increased. The study reveals that the RBC, WBC, haemoglobin and PCV values were considerably increased in cadmium and vitamin E treated chicks as compared to only cadmium treated chicks. These values were found nearest to control level in comparison to cadmium treated chicks. Hence, maximum protection by vitamin E was observed in this study.

Table 01- Antioxidant effect of vitamin E on haematological parameters in cadmium treated chicks.

Group	RBC ($10^6/\text{mm}^3$)	WBC ($10^3/\text{mm}^3$)	Hb (g/dl)	PCV (%)	S/L
Cadmium	$1.94 \pm .07$	11.73 ± 0.91	5.87 ± 0.73	14.25 ± 0.48	**
Cadmium + Vitamin E	3.70 ± 0.20	15.93 ± 1.3	11.13 ± 0.59	27.82 ± 0.148	**
Control	3.97 ± 0.09	19.43 ± 0.76	11.92 ± 0.82	29.91 ± 0.52	**

Results are expressed as mean $\pm$ SE. ** indicates significant at $p < 0.05$

Analysis of variance (ANOVA) was significant among all these groups. (RBC 187.57 $p < 0.05$, (WBC 88.08 $p < 0.05$, Hb 236.07 $p < 0.05$ and PCV 9.47 $p < 0.05$).

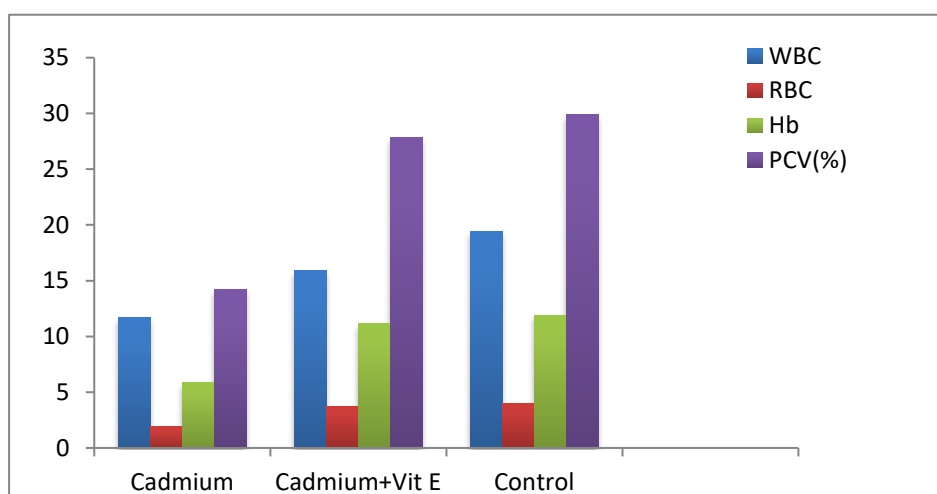


Figure 01- Antioxidant effect of vitamin E on haematological parameters in cadmium treated chicks.

The Cd is a trace element which is nonessential for human population and highly toxic to animals and plants [25]. It is released in cigarette smoke and industrial waste, which leads to environmental contamination [26]. The annually increasing concentration of Cd in air, water, and soil is a matter of great concern for the life on earth. Therefore, the present study was designed to explore the possible toxic effect of cadmium on haematological parameters in laboratory chicks (*Gallus gallus domesticus*) and its amelioration with vitamin E. Present study reveals that the Cd exposure induced haematological changes in chicks as evidenced by significantly decrease ($p < 0.05$) in RBC, WBC, Hb and PCV values. Cd exposition has induced anaemia which was

evidenced by the reduction of haemoglobinemia, the erythrocyte numeration and the haematocrit in exposed birds. Anaemia is a major haematotoxic effect following long-term exposure to Cd in human [27, 28] and laboratory animals [29, 30, 31].

As previously shown in rats, mice and broilers [32, 33, 34, 35, 36, 37] the anaemia is due to haemolysis due to Cd induced erythrocyte membrane damage. Red Blood Corpuscles (RBC) decreases in cadmium fed chicks while they increase in vitamin E treated chicks. The decreased red blood cell number following exposure to cadmium could be a result of haemolysis or destruction of red blood cells [38]. Anemia is an important manifestation of cadmium toxicity, cadmium induced anemia has been

attributed to an impairment in the synthesis of erythropoietin, a hormone whose function is to promote formation of the red blood cells [39].

Haemoglobin is the oxygen carrying component in blood of chicks and its concentration can be used as a good indicator of anaemia [40]. Hb percent in Cd treated chicks significantly reduced compared with control group, while it increased in vitamin E served groups in present study. The decreased haemoglobin in experimental chicks exposed to cadmium could thus be an indication that anaemic condition occurred in chicks during exposure. Decrease in RBC count and Hb levels may be due to the inhibition of erythropoiesis, haemolysis or increased rate of erythrocyte destruction in the hematopoietic organ [41].

Additionally, some authors [34] have also suggested that the impairment of iron absorption by Cd would lead to decrease the haemoglobin synthesis and would contribute to anaemia. The decrease in hemoglobin was also found in rabbits poisoned by lead [42], in mice exposed to cadmium chloride [43], in rats exposed to cadmium chloride [44]. White Blood Corpuscles (WBC) also decreased in cadmium fed chicks. This number of WBC increased in vitamin E treated chicks. The white blood cells respond to various stressors including infection and chemical irritants [45]. Thus the decreasing or increasing numbers of white blood cells are a normal reaction to a chemical such as cadmium, demonstrating the effect of immune system under toxic conditions. The decreased number of WBCs (leucopenia) may be the result of bioconcentration of test material in kidney and liver [46].

Haematocrite is used to determine the ratio of plasma to corpuscles in blood as well as the oxygen-carrying capacity of blood [47]. Decreases in number of red blood cells also decrease the amount of space they occupy, resulting in a lower haematocrite. The significant decrease in the PCV in present study may be due to anaemia, however this PCV value increased in vitamin E treated chicks. In present study PCV was significantly reduced in cadmium treated chicks as compared with control group.

Antioxidant is a type of molecules that neutralize harmful free radicals, produced through a chain of reactions [48], that damage living cells, spoil foods, degrade materials such as rubber, gasoline, lubricating oil. Antioxidants terminate these chain reactions through removal of free radical intermediates and

inhibition of other oxidation reactions [49]. Protective role of vitamin E against the toxicity of oxidants may be due to the quenching of hydroxyl radicals [50]. Vitamin E is an important antioxidant mainly located in cell membranes, the intramuscular vitamin E administration appears in present study as very effective against Cd induced changes. The vitamin C and vitamin E have successfully prevented anaemia in Cd exposed chickens and these findings are in agreement with previous data [35, 51, 52].

CONCLUSION

Ultimately, it may be concluded from the results of present study that cadmium causes haematotoxicity and severe anemia in chicks. These toxicological effects or haemato toxicity may be ameliorated by the administration of vitamin E in chicks as well as in other animals.

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