



## AMELIORATING EFFECTS OF VITAMIN E ON BLOOD PARAMETERS IN CHRONIC TOXICITY INDUCED BY CHROMIUM IN LABORATORY CHICKS

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### ABSTRACT

Experiments was conducted to study the role of vitamin E on haematological parameters in chromium induced toxicity in laboratory chicks. Analysis of normal haematological parameters of chicks is essential for the diagnosis of various pathological and metabolic disorders. It can be used as diagnostic tool in order to assess the health status of an individual and/or a flock. Haematological changes are commonly used to determine the body status and to assess the impact of environmental, nutritional and pathological stresses. Developing chicks (100±20 gm body weight, 2-3 weeks old) were used as experimental animals. Blood samples of chicks were collected and analyzed after experiment. Total Red Blood Cell count (RBC), total White Blood Cell count (WBC), Haemoglobin concentration (Hb) and Packed Cell Volume (PCV) were assessed. All these hematological parameters showed significant decrease in chromium treated group as compared to vitamin E administered group. Supplementation of vitamin E resulted in marked improvements in haematological parameters in co-treated group. The treatment of vitamin E normalized these haematological values up to the control level, signifying its protective effect in hexavalent chromium induced toxicity.

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### INTRODUCTION

Haematological parameters provide valuable information on the immune status of animals (Kral and Suchy, 2000). Such information, apart from being useful for diagnostic and management purpose, could equally be incorporated into breeding programmes for the genetic improvement of chickens. The hexavalent form of chromium has toxic effects on birds as it promotes the early aging process, reduces hatching ability and effects liver also (Asmatullah *et al.*, 1999). It also causes malformation or fetal death and leads to neural deformities. It has damaging effects on DNA and leads to mutation. It affects the function of gastrointestinal micro flora on chronic exposure to high dosages (Upreti *et al.*, 2004).

Cr (VI) is a global problem, having genotoxic effects in human beings. It is one of the top sixteen toxic pollutants which is an ever increasing threat to the environment. It is released during many industrial processes. Extensive experimental evidence indicates that chromium (VI) compounds are potent toxic and carcinogenic agents. (Leonard and Lauwerys, 1980; Doll, 1981; Furst and Haro, 1969; Mackison, *et al.*, 1982; Fradkin, *et al.*, 1975 and Sugiyama, *et al.*, 1986a and Sugiyama, *et al.*, 1986b).

Because of its carcinogenic characteristics for humans, it has been classified by International Agency of Research on cancer into Group 1 and by U.S.E.P.A. into Group A for causing lung cancer (Gardea-Torresdey, *et al.*, 2000; U.S. Environmental Protection Agency, 1999 and International Agency for Research Monographs, 1990).

In an organism's cells the important reason of the mutagenic activity of Cr (VI) is its oxidation properties. It can easily pass through the cellular membrane and oxidizes its constituents which results in metabolic reduction first to meta stable Cr (V), than to Cr (III). Migration of chromium metabolite complexes to nuclei and their interaction with DNA cause negative effect. A study shows that relatively long-lived chromium (V) species formed during reduction of chromium (VI) induce DNA single-strand breaks by generation of active oxygen radicals. (Kawanishi, *et al.*, 1986).

The importance of haematological and biochemical parameters as diagnostic tools and physiological indicators in birds has been documented (Perelman, 1999; Harr, 2002; Hauptmanova *et al.*, 2006). However, these parameters are greatly affected by sex, age and season (Fudge, 2000; Kececi and Col, 2011). Haematological and biochemical values were reported in many species of birds, particularly African and Asian reared chicken during summer season (Simaraks *et al.*, 2004; Pampori and Iqbal, 2007; Ladokun *et al.*, 2008; Melesse, 2011). In the other hand, many researchers have evaluated normal haematological

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parameters of industrial and commercial hybrid chickens (Meluzzi *et al.*, 1992; Talebi *et al.*, 2005; Abdi-Hachesoo *et al.*, 2011).

Among the various nutrients required by the poultry, vitamin E and selenium play a vital role in the development and maintenance of the defense system (Marsh *et al.*, 1982). Vitamin E and selenium are the integral part of body's natural antioxidant system and have synergistic action. It is well known that vitamin E is the most important exogenous antioxidant and protects cells from various oxidative damage (Dean and Cheeseman, 1987; Sandy, *et al.*, 1988; Summerfield and Tappel, 1984; Lieber, *et al.*, 1986; Ames, 1983). There is considerable evidence that vitamin E inhibits various oxidative steps in carcinogenesis (Ames, 1983; Borek, *et al.*, 1986; Rander and Kennedy, 1986).

Vitamin E is capable of reducing the levels of chromate-induced DNA single-strand breaks (Sugiyama, *et al.*, 1987). Thus it is important to elucidate the effect of vitamin E on the cellular damage induced by chromium (VI) compounds. In the present study we have extended these preliminary findings by examining the effect of vitamin E on haematological parameters (WBC, RBC Hb and PCV) in chromium fed laboratory chicks. Our findings illustrate that vitamin E can suppress chromium-induced toxicity, possibly through its ability to scavenge free radicals.

## MATERIAL AND METHODS

18 newly hatched domestic chicks were 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 purchased from the local market and tap water *ad libitum*. Healthy male and female chicks (body weight 100±20 gm) were used in present study.

The selected chicks were divided into three groups (A, B and C) randomly, each containing 6 chicks. Chicks of group A were administered with sub lethal chronic dose of potassium dichromate ( $K_2Cr_2O_7$ ) (5 mg/100 gm body weight) by gavage on each alternate day for 30 days. Chicks of group B were treated with potassium dichromate ( $K_2Cr_2O_7$ ) as chicks of group A but also administered with vitamin-E (intramuscularly) (0.5 IU/100 gm body weight) on each alternate day for 30 days. Chicks of Group C were administered with saline only to serve as control. Blood samples were collected from the wing vein using 3ml disposable syringe than 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.

**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

Table 1 shows the haematological values of different parameters as obtained in present study. Haematological parameters RBC, WBC, Hb and PCV showed significant ( $p < 0.05$ ) decrease in chromium supplemented chicks as compared to control group. While, co-treated with chromium and vitamin E, the value of these parameters increased. The study reveals that the leucocytes, haemoglobin and PCV values were considerably increased in chromium and vitamin E treated chicks as compared to chromium treated chicks. RBCs count slightly increase in co-treated group.

It was also observed that haemoglobin (Hb) value increase in co-treated groups in comparison to chromium administered chicks. Packed Cell Volume (PCV) was also observed increased in chromium and vitamin E treated chicks. These values were found nearest to control level in comparison to chromium treated chicks. Hence, maximum protection by vitamin E was observed in this study.

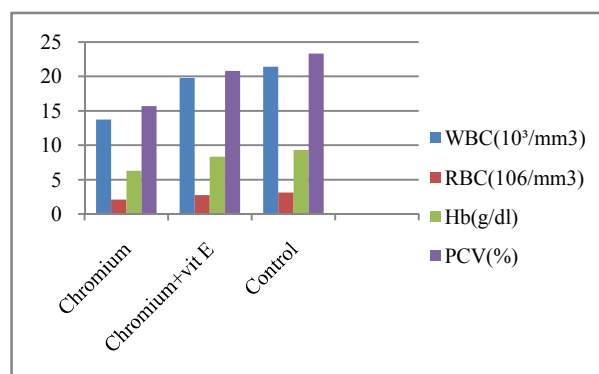
**Table 1** Protective effect of vitamin E on hematological parameters in chromium treated chicks.

Treatment	WBC ( $10^3/mm^3$ )	RBC ( $10^6/mm^3$ )	Hb (g/dl)	PCV (%)	S/L
Chromium	13.73 ± 1.44	2.08 ± 0.13	6.27 ± 0.37	15.67 ± 0.93	**
Chromium+Vitamin E	19.77 ± 0.85	2.77 ± 0.18	8.32 ± 0.55	20.79 ± 1.38	**
Control	21.40 ± 0.53	3.10 ± 0.05	9.32 ± 0.15	23.31 ± 0.38	**

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

WBC – White blood cell, RBC – Red blood cell, Hb – Hemoglobin, PCV – Packed cell volume

Analysis of variance (ANOVA) was significant among all these groups. (WBC 38.44  $p < 0.05$ , RBC 47.64  $p < 0.05$ , Hb 48.33  $p < 0.05$  and PCV 46.86  $p < 0.05$ ).



**Figure 1** Protective effect of Vitamin E on haematological parameters in Chromium treated chicks

## DISCUSSION

Present observations showed significant decrease in the number of RBC, WBC, PCV and Hb contents in chromium treated chicks. Treatment with vitamin E increases the number of RBC, WBC, PCV and Hb contents. Previous studies noted decrease in hematological parameters following Cr-administration i.e. in broiler (Kumari, *et al.*, 2013), in rats (Kim, *et al.*, 2004; Balakrishnan, *et al.*, 2013) and fish (Shaheen and Akhtar, 2012). Reduced hemoglobin concentration and PCV values in chromium treated group were due to the intestinal haemorrhage resulting from the liberation of second generation merozoites which caused sloughing of intestinal mucosa with discharge of large amount of blood (Nayak and Rai, 1985). Decrease in RBC, Hb and PCV level indicate

anemia resulting due to iron deficiency and its decreased utilization for Hb synthesis, so Hb concentration also decreases.

When Cr(VI) was inhaled or administered intratracheally, intraperitoneally, or intravenously, much of the chromium in the blood (25 to 70%) was taken up by RBCs (Sayato, *et al.*, 1980; Weber, 1983; Wiegand, *et al.*, 1984; Edel and Sabbioni, 1985; Minoia and Cavalleri, 1988; Gao, *et al.*, 1993). As the erythrocyte to plasma ratio of total chromium increases with increasing hexavalent chromium concentration, Corbett, *et al.*, (1998) proposed that the reductive capacity of erythrocytes was much greater than that of plasma and that the reduction rate of hexavalent chromium in erythrocytes was greater than the rate of uptake from plasma.

Another possible reason could be that the Cr can cross the red blood cell membrane easily and accumulate in it, thereby leading to DNA protein cross linking and thus occurrence of anemia. Another reason could be that Cr has ability to bind to beta chain of hemoglobin so no hemoglobin available for heme synthesis leading to anemia (Adjroud, 2010).

In the present study, leukocyte count was also decreased in chromium treated birds compared to control group. Previous studies report decreases in leukocyte count following Cr administration in the rats (Balakrishnan, *et al.*, 2013). Decrease in leukocyte count could be due to in activation of immune response by chromium. Another opinion could be that leucopenia could be due to ability of chromium to cross membrane through active transport and remain there till the life of the cell leading to depletion of leukocytes. Cr-administration leads to apoptosis of leukocytes leading to leucopenia. Chromium when comes in contact with leukocytes causes peroxidation and inhibits trans membrane potential of mitochondria of lymphocytes (Adjroud, 2010).

Present study showed decrease in haematological parameters after chromium treatment. Co-treatment with antioxidant (vitamin E) increased all these haematological parameters nearest to control level. This might be due to the supplemented vitamin E increase in oxygen carrying capacity of hemoglobin. Therefore, vitamin E is an important antioxidant which protects the organs from harmful effects of heavy metals.

## CONCLUSION

The toxic effects of Cr and its amelioration with vitamin E on haematological parameters were profiled in this study. It may be concluded that chromium caused the formation of free radicals in the blood by reducing the antioxidant indices. However, vitamin E supplementation to chromium fed chicks exhibited therapeutic effects indicating its protective antioxidant property. Thus, present study confirmed the role of vitamin E as a scavenger of free radicals, probably preserving haematological values in chromium treated laboratory chicks.

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