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Protective Effect of Vitamin E on Haematological Parameters in Chronic Toxicity of Hexavalent Chromium in Laboratory Chicks

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ABSTRACT

Analysis of blood parameters is relevant to risk evaluation of alternations of the haematological system in humans and animals. Haematological parameters have been associated with health indices and are of diagnostic significance in routine clinical evaluation of the state of health of any individual. Present study was carried out to ascertain the antioxidant properties of vitamin E on haematological parameters caused by hexavalent chromium - Cr(VI) in laboratory chicks. Developing chicks (Croiler, body weight 100±20 gm) were used as experimental animals. Blood samples were collected from wing veins of chicks and analyzed after experiment. The haematological profile revealed a significant ($p<0.05$) reduction of Total Red Blood Cell count (RBC), total White Blood Cell count (WBC), Haemoglobin concentration (Hb), Packed cell volume (PCV) and Mean corpuscular hemoglobin concentration (MCHC) in toxin group compared to control group. However, the group of chicks treated with chromium and vitamin E alternatively, recorded significant increase in all these blood parameters indicating the protection offered by vitamin E as an ameliorating antioxidant against chromium toxicity.

Keywords: Haematological parameters, hexavalent chromium [Cr(VI)], vitamin E, antioxidant.

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INTRODUCTION

Chromium is one of the major environmental toxicants that affect human health. It is one among the eight most common pollutant heavy metals listed by the Environmental Protection Agency[1]. It is a naturally occurring element found in rocks, volcanic dust and gasses, soils as well as plants and animals. This transition metal can exist in various oxidized forms ranging from −2 to +6. The three main oxidation forms of chromium, commonly found in the workplace and general environment, are chromium (0), chromium (III), and chromium (VI). Chromium (0) is the metal chromium, a steel-gray solid with a high melting point usually used for making steel and other alloys. Chromium (III) and chromium (VI) compounds are widely used industrially in stainless steel production, welding, electroplating, leather tanning, production of dyes and pigments, and wood preservatives[2,3].

It is well known that oral intake along with food and water is the major route of exposure to chromium for the general population. It has been reported that the chromium (VI) form appears to be 10-100 times more toxic than the chromium (III) form when both are administered by the oral route[4]. It is an establish fact that chronic diseases affect the blood cells adversely. Exposure to chromium, the transition element found in many compounds of earth's crust, leads to various health hazards including cancer, dermatitis, damage of liver and kidneys, and alternations in haematological parameters. Toxic effect of chronic exposure to chromium at low environmentally relevant dose is recently recognized and less studied.

Chromium (VI) can easily enter the cell than chromium (III) through SO_4^{2-} and PO_4^{2-} channels. After entering the cell, chromium (VI) undergoes a chain reaction with production of chromium intermediates such as chromium (V) and chromium (IV) by cellular reductants such as ascorbic acid and riboflavin, glutathione, and serum protein[5]. The reduced product binds to intracellular proteins, resulting in an elevation of total chromium in the blood cell for several weeks. During this reduction process, chromium produces reactive oxygen species and generates oxidative stress. This in turn is responsible for defective haematopoiesis[6].

It has been reported that chromium (VI) is considered as a toxic transition heavy metal and a potent industrial hazard that causes severe damage to a variety of tissues and organs including the reproductive system[7-10]. Most of the studies on

toxic effects of chromium involve a dose much higher than the amount of chromium available in drinking water and within the permissible limit set by the World Health Organization[11].

Vitamin E (α-tocopherol) is a biological antioxidant, soluble in fat[12], which inhibits the oxidation of long chained unsaturated fatty acids of the cell membrane[13,14]. Unsaturated fatty acids react with oxygen, and form superoxide, peroxide and hydroperoxides[15]. These free radicals cause cell damage by disturbing the metabolism and structure of the biological membranes of those organs that contain excessive amount of unsaturated fatty acids[16]. Vitamin E inhibits the effects of hydrogen protons and free radicals by saturating them, and so inhibits auto oxidation[13,15]. It has been reported that lipid peroxidation is stopped in chicks fed with a vitamin E supplemented diet[17]. It has also been proposed that vitamin E inhibits the oxidation of unsaturated fatty acids such as linoleic acid on the erythrocyte membrane[15], and the deficiency of this vitamin increases the hemolysis of red cells[18,19].

Although much research investigating the ameliorating effect of vitamin E on the chromium toxicity has been done on humans and various animals, little work has been attempted on animals undergoing rapid growth, such as broilers[20]. Therefore, in this study, we aimed to investigate the protective effects of vitamin E in the toxicity caused by chromium on a number of haematological parameters in laboratory chicks.

OBJECTIVE OF STUDY

The objective of the present work was to study the toxic effects of hexavalent chromium in the form of potassium dichromate ($K_2Cr_2O_7$) on haematological parameters and its treatment with vitamin E. In this study we hypothesize that vitamin E act as an antioxidant against toxicity induced by different heavy metals and it may play an important role to reduce chromium induced toxicity. To prove this fact, we studied the protective effects of vitamin E against hexavalent chromium toxicity on haematological parameters. However, to strengthen this work, further studied are required to clarify the action mechanism of vitamin E as a therapeutic agent. This study is expected to enhance our understanding of harmful effects of chromium toxicity and the effectiveness of vitamin E to reduce chromium health hazards. An attempt was made to evaluate the beneficial effect of vitamin E on chromium mediated toxicity in order to study the role of vitamin E in the amelioration of the chromium intoxication.

MATERIALS AND METHODS

Chemicals – Hexavalent chromium - Potassium dichromate ($K_2Cr_2O_7$) was procured from Glaxo (India). Vitamin E (α-tocopherol) was purchased from Sigma Chemical Co. (St. Louis, Mo, USA). All the reagents and chemicals used in this study were of analytical grade and highest purity procured from standard commercial sources in India.

Animals - The experiment was carried on Domestic chicks - Croiler Chabro (Gallus *gallus domesticus*). Newly hatched chicks were purchased from the Uttarakhand Village Poultry Project (State Govt. Poultry Farm), Bin, Pithoragarh (Uttarakhand). Selected all chicks were maintained and acclimatized according to the laboratory conditions. The animals were housed in battery cages under laboratory conditions at existing room temperature and relative humidity. They were fed on commercial food (Starter, Grower and Finisher) purchased from the local market and tap water *ad libitum*.

Healthy male and female chicks (approximately 2-3 weeks old, body weight 100±20 gm) were used in present study. All protocols were approved by the Institutional Animal Ethics Committee (IAEC), Department of Pharmaceutical Science, Bhimtal, Kumaun University, Nainital and the member secretary, CPCSEA, Ministry of Environment, Forest and Climate Change, Government of India (Protocol No.- KUDOPS/89). The animals were kept under standard conditions throughout the experiment to reduce the error. Minimum number of animals was used to obtain reliable results.

Experimental Treatments - The selected chicks were divided into three groups (A, B and C) randomly, each containing at least 6 chicks. Chicks of group A were administered with sublethal dose of potassium dichromate ($K_2Cr_2O_7$) (5 mg/100 gm body weight) dissolved in distilled water 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 purely control.

Haematological studies – The fresh blood samples were collected from the wing vein using 3ml disposable syringe and directly transferred into labeled test tubes containing EDTA, as anti – coagulant, kept in an ice box, using icepacks and transferred to the laboratory for measuring the hematological parameters.

The haematological indices determined included Haemoglobin concentration (Hb), White blood cell count (WBC), Red blood cell count (RBC), Packed cell volume(PCV) and Mean corpuscular hemoglobin concentration (MCHC). Blood cells counts were done by Neubauer double haemocytometer using Haem's and Turek's solution as respective diluting fluids. Haemoglobin percentage was measured by cyanomethemoglobin method. PCV and MCHC were determined by Wintrobe's hematocrit tube method.

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 01 shows the haematological values of different parameters as obtained in present study. Hematological parameters Hb, WBC, RBC, PCV and MCHC showed significant ($p<0.05$) decrease in chromium supplemented chicks (Group A) as compared to control group (Group C). While, in the group co-treated with chromium and vitamin E (Group B), the value of these parameters increased. These values were found nearest to control level in comparison to chromium treated chicks.

Table 1: Protective effect of vitamin E on haematological parameters in hexavalent chromium treated laboratory chicks.

Treatments	Hb (g/dl)	WBC (10^3/mm^3)	RBC (10^6/mm^3)	PCV (%)	MCHC (mg/dl)
Cr(VI)	6.27±0.37*	13.73±1.44*	2.08±0.13*	15.67±0.93*	39.18±0.26*
Cr(VI) + Vitamin E	8.32±0.55**	19.77±0.49**	2.77±0.18**	20.79±1.38**	40.56±0.51**
Control	9.32±0.15	21.40±0.53	3.10±0.05	23.31±0.38	40.54±0.65
F- ratio	48.33	38.44	47.64	46.86	1.79^{NS}

Results are expressed as mean ± SE.

* Significantly different (p< 0.05) when compared with control chicks.

** Significantly different (p< 0.05) when compared with chromium treated chicks.

Analysis of variance F (ANOVA) was significant for Hb, WBC, RBC and PCV. NS = non significant.

Hb – Haemoglobin, WBC – White blood cell, RBC – Red blood cell. PCV – Packed cell volume, MCHC - Mean corpuscular hemoglobin concentration.

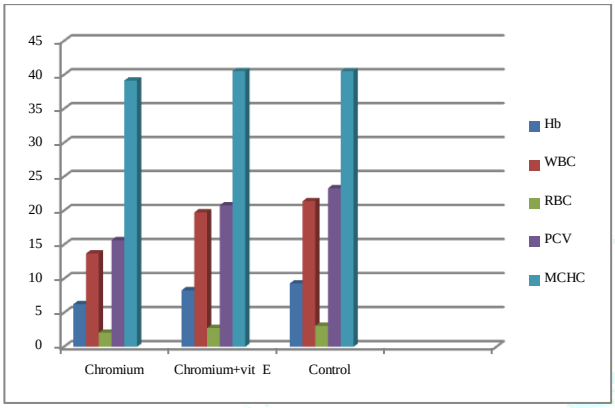

Figure 1: Protective effect of Vitamin E on haematological parameters in Chromium treated chicks.

DISCUSSION

Blood is the most important tissues, in which changes in metabolic processes are going on, so abnormal alteration in blood parameters are the reliable indicators of toxic effects of drugs, chemicals and diseases. Haematological parameters are usually related to health status and are of diagnostic importance in clinical evaluation of the state of health. The haematological parameters tested in current study include haemoglobin concentration, red and white blood cell counts, packed cell volume and Mean corpuscular hemoglobin concentration showed significant (p<0.05) decrease in Cr treated chicks as compared to vitamin E and control group.

Present study reveals that chromium induces anemia type of condition with decreased RBC and WBC count and haemoglobin concentration. Earlier reports showed that chromium exposure to rats resulted in reduction of RBC count and haematocrit value along with a decrease in haemoglobin concentration. The haemoglobin is the oxygen carrying pigment present in the red blood cells of vertebrates and synthesized in the immature erythrocytes in the bone marrow. The function of haemoglobin is the transport of oxygen from the lungs to the tissues and of carbon dioxide in the opposite direction and it is also responsible for stabilizing the oxygen pressure in the tissues.

The erythrocytes are transporter of haemoglobin. They also contain a large quantity of carbonic anhydrase. All living bodies have a special system for combating the different infectious and toxic agents which is composed of the blood leukocytes and tissue cells derived from the leukocytes. These are the mobile units of the body's protective system.

The decrease in haemoglobin appears to be due to inhibition of its biosynthesis by decreasing the succinyl pool as well as glycine pool[21]. Another simplest explanation for this diminution in haemoglobin concentrations could be probably due to structural alteration of heame which disturbs haemoglobin synthesis and also to the inhibition of the enzyme system involved in the synthesis of haemoglobin[22]. This decrease may also be due to binding of Cr(VI) to beta-chain of haemoglobin in the erythrocyte[23], explaining the depleted concentrations of heamoglobin.

A significant decrease in the RBC count was observed because **Cr(VI)** can penetrate rapidly through the membrane of erythrocyte and enter the cell and accumulates in the erythrocytes[24-26]. 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[27-32]. As the erythrocyte to plasma ratio of total chromium increases with increasing hexavalent chromium concentration, Corbett et al.[33] 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.

Cr(VI) taken up by RBCs undergoes reduction to the trivalent form with the help of reduced glutathione[29] and complexes with Hgb and other intracellular proteins that are sufficiently stable to retain chromium for a substantial fraction of the RBC lifetime[34]. This was confirmed by the result of an experiment where $K_2Cr_2O_7$, a hexavalent chromium compound, introduced into plasma and reconstituted whole blood from three individuals was found to be readily reduced to Cr(III) in the concentration range of 100–1,000 µg Cr(VI)/L[33]. Excess trivalent chromium in the RBC is sequestered until cell death[34,35]. Over time, the RBC-associated chromium appears to be transferred to the spleen as a result of scavenging aging RBCs from the blood. The red blood cell chromium is currently considered as the best indicator of hexavalent chromium exposure as reported by Costa et al.[36]

In this present study, along with decreased RBC count, there was also decrease in WBC count. This might be due to generalized injury caused to the hematopoietic stem cells by chromium treatment[37]. Similar disruption in hemopoitic mechanisms was observed by Adjroud[38] after potassium dichromate exposure in both male and female rats. These findings are in co-occurrence with the findings of the Geetha et al.[39] and Adjroud[38]. The reduction in WBC could be due to contact of **Cr(VI)** with biological compounds which lead to peroxidation of these compounds which were present in the cell or on its surface. It effect, some negative changes such as cells membrane damage due to peroxidation of unsaturated

fatty acids or inhibition of both mitochondrial trans-membrane potential in rat lymphocytes[39].

Reduced hemoglobin concentration, PCV and MCHC 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[40]. Decrease in RBC, Hb, PCV and MCHC level indicate anemia resulting due to iron deficiency and its decreased utilization for Hb synthesis, so Hb concentration also decreases. Previous studies also noted decrease in hematological parameters following Cr administration i.e. in broiler[41], in rats[42-43] and fish[44].

Hematological parameters RBC, WBC, Hb, PCV and MCHC concentration studied in the present investigation showed a significant increase in vitamin E therapeutic group. Vitamin E acts as an antioxidant which protects cells against toxic effects of chromium. Antioxidants are molecules, which interact with free radicals and terminate the chain reaction before vital molecules are damaged. They donate an electron to stabilize a free radical. Antioxidants have long been known to reduce the free radical mediated oxidative stress caused by elements and compounds in the environment[45,46.] Vitamin E is synthesized by plants and is an antioxidant that protects all membranes and other fat-soluble parts of the body, such as low-density lipoprotein cholesterol, from damage. Vitamin E is absorbed from the intestine through lymph. It circulates through the body plasma in associations with beta-lipoprotein.

Vitamin E is a natural component of the membrane lipid bilayer and thus helps to maintain membrane stability[47]. The molecular and cellular effects of vitamin E have been explained either by acting as an antioxidant preventing damage to membranes or proteins and regulating their activity by specifically scavenging reactive oxygen species[48,49] or by interacting and regulating specific enzymes and influencing cellular structure such as membrane and lipid domains[50]. Vitamin E may effectively minimize lipid peroxidation in biological systems[51-53]. Vitamin E is the major chain-breaking antioxidant in body tissues and is considered the first line of defense against lipid peroxidation, protecting cell membranes an early stage of free radical attack.

Present study showed decrease in haematological parameters after chromium treatment. Co-treatment with antioxidant (vitamin E) increased 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 chromium.

CONCLUSION

From the present study it may be concluded that the haematological profile showed a significant decrease in the overall mean values of Hb, RBC, WBC, PCV, MCHC at significant ($p<0.05$) level in comparison with control chicks. While, the chicks supplemented with vitamin E along with chromium shows significant increase in all these hematological parameters. Hence, it can be concluded that the progressive toxic effect of hexavalent chromium can be moderately reduced by supplying vitamin E in laboratory chicks.

CONFLICT OF INTEREST

There is no conflict of interest.

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