RESEARCH ARTICLE

Effect of Vitamin E and Selenium on Hematological Parameters in Sub-acute Toxicity of Hexavalent Chromium in Broiler Chick

Rashmi Rekha Kumari[1], Pankaj Kumar[2], TK Mondal[3]

[1] Department of Pharmacology and Toxicology, Bihar Veterinary College, Patna, Bihar, India

[2] DLFM, ICAR-RCER, Patna, Bihar, India

[3] Department of Pharmacology and Toxicology, West Bengal University of Animal and Fishery Sciences, Kolkata

Correspondence to:
Rashmi Kumari
(rrkvet@gmail.com)

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ABSTRACT

Background: Chromium (Cr), a naturally occurring heavy metal, exists in many states, but the trivalent Cr (III) and hexavalent Cr (VI) forms are the most common in the environment. Acute and chronic toxicity in both animal and man are mainly caused by Cr (VI) compounds. Studies on hematological parameters in sub-acute toxicity of potassium chromate in broiler chicks are limited.

Aims & Objective: The study was envisaged to evaluate the protective effect of vitamin E and Selenium on haematological parameters in sub-acute oral toxicity of potassium chromate [Cr (VI)] in broiler chicks.

Materials and Methods: Potassium chromate (K_2CrO_4) used as test compound and Vitamin E and Se powder as Tocosel® contained 5 g of Tocopherol acetate and 30 mg of sodium selenite per 10 gm. LD50 was calculated before initiating the experiment by graphical probit analysis method. Experiment was conducted in randomly divided four equal groups (n=6) of day old chicks. Chicks of each group were maintained up to the age of 45 day and sacrificed on 46th day. Chicks of control group (T1) were provided only with basal diet and drinking water. While groups T2 and T3 was fed different concentrations of K_2CrO_4 and T4 as treatment group supplemented with Vitamin E and Se.

Results: LD50 of K_2CrO_4 in broiler chick was calculated as 277.95 mg/kg. Feeding potassium chromate [Cr (VI)] in group T2 (1/5th of LD50) and T3 (1/3rd of LD50) for 30 days, decreased haemoglobin percentage, haematocrit values, total erythrocyte count and total leucocyte count while increased the clotting time in a dose dependent manner. In group T4 chicks fed 1/3rd of LD50 of K_2CrO_4 and simultaneous treatment of Vitamin E and Se, normalised these haematological values, signifying its protective effect in sub-acute hexavalent chromium toxicity produced by potassium chromate.

Conclusion: It may be concluded that above mentioned changes might have reduced erythrocyte number, leucocyte number, haemoglobin value and increased clotting time. Vitamin E and Se containing glutathione peroxidase are among the principle in vivo chain breaking antioxidants, thus had protective effect.

KEY WORDS: Hematology; Hexavalent Chromium; Selenium; Vitamin E

INTRODUCTION

Chromium (Cr), a naturally occurring heavy metal, exists in many states, but the trivalent $Cr^{(III)}$ and hexavalent $Cr^{(VI)}$ forms are the most common in the environment.[1] $Cr^{(III)}$ act as essential trace element of body in animal and human and has nutraceutical importance while $Cr^{(VI)}$ present in the environment is mostly man mad through industrial emission. Acute and chronic toxicity in both animal and man are mainly caused by $Cr^{(VI)}$ compounds.[2] The soluble hexavalent chromium $Cr^{(VI)}$ is an environmental contaminant widely recognized as carcinogen, mutagen, and teratogen toward humans and animals. The wide distribution of this contaminant in the environment is the result of its extensive exploitation to produce stainless steel, wood treatment products, tanning of leather or pigments.[3,4] The fate of chromium in the environment is dependent on its oxidation state. The reduction of $Cr^{(VI)}$ to $Cr^{(III)}$ results in the formation of reactive intermediates leading to oxidative tissue damage and cellular injury.[5] Exposure of rats at different concentration of aerosol of chromium trioxide decreased total erythrocyte count, haematocrit value, haemoglobin%.[6] Feeding of $Cr^{(VI)}$ in drinking water led to reduction in lymphocyte, haematocrit value, and haemoglobin value in mice. However, studies on haematological parameters in sub-acute toxicity of potassium chromate in broiler chicks are limited.

Therefore, the present study was undertaken to evaluate the effect of potassium chromate [$Cr^{(VI)}$] at different fraction of LD_{50}, on haematological parameters in broiler chicks. Since Vitamin E and Selenium (Se) have protective effect against oxidative stress[7], kidney toxicity[8], liver damage[9], protective effect of Vitamin E and Se was evaluated.

MATERIALS AND METHODS

Chemicals and Drugs

Potassium chromate (K_2CrO_4) of E. Merck (India) limited was used as test compound. Vitamin E and Se powder was (Tocosel®) supplied by M/S Nandan remedies 311/8, Nagendra road, Kolkata. Each 10 g of Tocosel® contained 5 g of Tocopherol acetate and 30 mg of sodium selenite.
Design of Experiment:

LD_{50} value of K_2CrO_4 in 3 weeks old broiler chicks (Strain- Vancobb) was calculated before initiating the experiment by graphical probit analysis method described.[10] Experiment was conducted using one day old healthy vaccinated broiler chicks. They were randomly divided into four equal groups (n=6) as T_1, T_2, T_3 and T_4. Chicks in group T_1 was considered as control while T_2, T_3 and T_4 were experimental groups. Chicks of each group were maintained up to the age of 45 day and sacrificed on 46th day. Chicks were fed with balanced broiler starter feed (Metabolic energy 2890 Kcal /Kg and crude protein 22.1%) for first 4 weeks followed by broiler finisher feed (Metabolic energy 2945 Kcal /Kg and crude protein 19.10%) for the remaining period of experimentation as per recommendation of (Bureau of Indian standards) BIS.[11] Fresh water and feed was supplied ad lib. From 15th to 45th day of age chicks of group T2 and T3 were fed 1/5th of LD_{50} dose and 1/3rd of LD_{50} dose of K_2CrO_4 (Cr VI), respectively for consecutive 30 days.

Chicks of group T_4 were treated with 1/3rd of LD_{50} dose of K_2CrO_4 along with Vitamin E + Se for the same duration (15th to 45th day of age). Vitamin E and Se powder was orally given @ 600 mg/kg body weight and initiated one week prior to the feeding of K_2CrO_4. Potassium chromate dissolved in water was fed by feeding syringe directly into ventriculus while Vitamin E and Se powder was fed after dissolving in (600mg Vitamin E and Se in 0.5 ml) olive oil directly into proventriculus. Chicks of control group (T_1) were provided only with basal diet and drinking water.

Hematological Study

Blood samples (3 ml) were collected from wing vein of chicks in each group on 15, 30 and 45 day of age for haematological study. EDTA (5%) was used as anticoagulant. Total erythrocyte count (TEC), Total leucocyte count (TLC)[12],

Table-1: Effect of Potassium Chromate alone and in Presence of Vitamin E and Se on Hemogram in Broiler Chicks on Different Days of Treatment after Consecutive Oral Administration for 30 days. (Mean ± SE, n=6)

Parameter	15 day				30 day				45 day			
	T1	T2	T 3	T 4	T 1	T 2	T 3	T 4	T 1	T 2	T 3	T 4
Hemoglobin (gm/L)	83.0^{a} ± 3.6	84.0^{a} ±3.0	83.0^{a} ± 3.3	82.0^{a} ± 2.5	83.4^{a} ± 2.9	86.0^{a} ± 1.8	87.0^{a} ± 1.6	85.0^{a} ± 1.8	90.0^{a} ± 2.8	79.0^{b} ± 2.9	70.0^{c} ± 2.9	92.0^{a} ± 2.7
PCV	0.26^{a} ±0.012	0.25^{a} ±0.016	0.25^{a} ±0.011	0.25^{a} ±0.015	0.26^{a} ±0.007	0.26^{a} ±0.006	0.28^{a} ±0.008	0.26^{a} ±0.007	0.30^{a} ±0.009	0.26^{b} ±0.016	0.22^{c} ±0.008	0.29^{a} ±0.007
Clotting Time (sec.)	150^{a} ±10.60	165^{a} ±9.01	170^{a} ±7.71	155^{a} ±8.74	155^{a} ±7.90	165^{a} ±7.07	250^{b} ±10.09	170^{a} ±9.71	160^{a} ±10.6	260^{b} ±10.08	395^{c} ±15.96	160^{a} ±7.58
TEC (10^{12}/L)	2.30^{a} ±0.15	2.16^{a} ±0.16	2.12^{a} ±0.14	2.15^{a} ±0.25	2.29^{a} ±0.18	2.59^{a} ±0.23	2.90^{a} ±0.25	2.54^{a} ±0.18	3.30^{a} ±0.19	2.50^{b} ±0.20	1.52^{c} ±0.09	2.99^{a} ±0.21
TLC (10^{9}/L)	18.10^{a} ±0.56	18.50^{a} ±0.26	18.00^{a} ±0.55	19.00^{a} ±0.49	20.00^{a} ±0.47	20.80^{a} ±0.78	21.00^{a} ±0.38	19.80^{a} ±0.54	22.40^{a} ±0.61	19.16^{b} ±0.58	17.00^{c} ±0.43	22.00^{a} ±0.45

The values with dissimilar superscript vary significantly (P <0.05)

Hemoglobin (Hb) level[13], haematocrit value (PCV) by Wintrobe hematocrit tube[14], Clotting time were estimated and expressed as SI unit[14].

Statistical Analysis

Mean and standard error were calculated and data were analysed using standard methods. [15] Differences at P<0.05 (at least) were considered to be significant.

RESULTS

LD_{50} of K_2CrO_4 in broiler chick was calculated as 277.95 mg/kg using probit analysis. From the perusal of table1, it is evident that on 15th day of age, there was no significant difference between different hemogram of control (T_1) and experimental groups (T_2, T_3 and T_4). On 30th day of age there was no change in Hb, PCV, TEC and TLC in chicks of experimental group as compared with control. But clotting time in chicks of T_3 group was significantly increased as compared to other group. On 45th day of age there was a significant decrease in Hb, PCV, TEC and TLC in chicks of both T_2 and T_3 groups in dose dependent manner as compared to control (T_1). Further, these parameters in group T_3 chicks were significantly lower compared to group T_2 chicks. But there were no significant difference in these parameters of group T_4 chicks as compared to control. Likewise clotting time in T_2 and T_3 groups were significantly increased compared to control (T_1). Clotting time in T_4 group was equivalent to normal control chicks (T_1).

DISCUSSION

Result shows that potassium chromate (Cr^{VI}) produced toxic effect on haematological parameters such as total erythrocyte count, total leucocyte count, haematocrit value, haemoglobin% and clotting time in broiler chicks in dose dependent manner after 30 day of consecutive oral treatment. Researcher[6] observed that exposure of rats at different concentrations of aerosol of chromium trioxide (Cr^{VI}) decreased TEC, PCV value and Hb% which is in consonance with the present findings. Feeding of Cr^{VI} in drinking water led to reduction in lymphocyte, PCV value, Hb value in mice.[16] Increase in clotting time indicates that potassium chromate produced liver damage. A series of in vitro and in vivo studies have demonstrated that chromium (Cr^{VI}) induces an oxidative stress through enhanced production of reactive oxygen species (ROS) leading to genomic DNA damage and oxidative deterioration of lipids and proteins. A cascade of cellular events occur following chromium (Cr^{VI}) induced oxidative stress including enhanced production of superoxide anion and hydroxyl radicals, increased lipid peroxidation and genomic DNA fragmentation, modulation of intracellular oxidized states, activation of protein kinase C, apoptotic cell death and altered gene expression.[17] Vitamin E is involved in removal of free radical and prevents their peroxidative effect on unsaturated lipid of membrane and thus help in maintaining integrity of membrane. Chromanol ring of tocopherols donates its phenolic hydrogen to reduce the free radical and is itself oxidised to the quinone form. In the Vitamin E and Se treated

group TEC, TLC, PCV, Hb value and clotting time were preserved, suggesting that oxidative damage is a participating mechanism in Cr (VI) toxicity on these functions.

CONCLUSION

It may be concluded that above mentioned changes might have reduced erythrocyte number, leucocyte number, haemoglobin value and increased clotting time. Vitamin E and Se containing glutathione peroxidase are among the principle in vivo chain breaking antioxidants, thus had protective effect.

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