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Jeevana Latha M
Assistant Professor, Department of Veterinary Pathology, College of Veterinary Science, PVNR TVU, Rajendranagar, Hyderabad, Telangana, India

Dr. Srikanth MK
Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science, PVNR TVU, Rajendranagar, Hyderabad, Telangana, India

Dr. Gopala Reddy A
Professor and University Head, Veterinary Pharmacology and Toxicology, College of Veterinary Science, PVNR TVU, Korutla, Karimnagar. Telangana, India

Dr. Anudeep Reddy M
Veterinary Pharmacology and Toxicology, College of Veterinary Science, PVNR TVU, Rajendranagar, Hyderabad, Telangana, India

Correspondence
Jeevana Latha M
Assistant Professor, Department of Veterinary Pathology, College of Veterinary Science, PVNR TVU, Rajendranagar, Hyderabad, Telangana, India

Haematological study in hexavalent chromium toxicity in female wistar rats and its progeny

Jeevana Latha M, Dr. Srikanth MK, Dr. Gopala Reddy A and Dr. Anudeep Reddy M

Abstract
An experiment was conducted to study the haematological profile in 120 female wistar rats in chromium toxicity. Rats were provided with feed and water *ad libitum*. The duration of experiment was for four months. There were six groups where as group I was the control group. Group II was provided with potassium dichromate @ 500ppm in drinking water orally for three months along with the basal diet, group III was treated as Vitamin C control, group IV as *Emblica officinalis* control, group V and VI were protective and ameliorative groups. The haematological profile revealed a significant (p<0.05) reduction of haemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC) and total leukocyte count (TLC) in toxin group. Group V and VI recorded mild to moderate results indicating the protection offered by ameliorating agents used in the feed (Vitamin C @100 mg/kg body weight and *Emblica officinalis*@ 2%).

Keywords: Chromium toxicity, female wistar rats, *Emblica officinalis*, Haematology, Vitamin C

1. Introduction
Chromium (Cr-VI) is extensively used in metallurgical processes and the chemical, chrome plating, pigment production, catalytic converters, asbestos brake lining, cement dust, tobacco and food additives, tanning textile, ceramic, glass and photographic industries (Sakhila *et al.*, 2008) [1]. Chromium occurs in each of the oxidation states from -2 to +6 but only the 0 (elemental metal form), +2, +3 and +6 states are common (Dayan and Paine, 2001) [2]. The problem of environmental pollution is one of the most burning topics and has been on the check list of almost all the nations. Among these environmental pollutants, heavy metals such as chromium received special attention world wide as they are widely distributed in nature and leads to wide spread occurrence of specific toxicological problem (Patra and Swarup, 2000) [3]. The availability of literature is very scanty on the usage of herbal drugs in ameliorating the toxic effects of chromium. Hence the present study was undertaken as a fore step.

2. Materials and methods
An experiment was carried at Laboratory Animal House, College of Veterinary Science, Rajendranagar Hyderabad to study the haematological profile in hexavalent chromium toxicity in female wistar rats and its progeny.

2.1 Experimental Design
After an acclimatization period of 2 weeks, 120 female wistar rats were divided into 6 groups of 20 rats in each and were maintained for 4 months (120 days) in the laboratory animal house of the Department of Veterinary Pharmacology & Toxicology with the following treatment schedule (Table 1).

Table 1: Experimental design-completely randomized design and its respective treatment schedules

Group	No of Rats	Type of treatment / diet
I (Control)	20	Basal diet
II (Toxin control)	20	Basal diet + Potassium dichromate @ 500 ppm in drinking water orally for 3 months
III (Vit C control)	20	Basal diet + vitamin C @ 100 mg /kg b.wt orally for 3 months.
IV(*Emblica officinalis* control)	20	*Emblica officinalis* powder given @ 2% in feed for 3 Months
V (Chromium VI + Vitamin C control)	20	Basal diet + Potassium dichromate @ 500 ppm in drinking water orally for 3 months + vitamin C @ 100 mg/kg b.wt orally for 3 months.
VI (Chromium VI + *Emblica officinalis*)	20	Basal diet + potassium dichromate @ 500ppm in drinking water orally for 3months + *Emblica officinalis* powder given @ 2% in feed for3months.

2.2 Blood collection

Blood samples were collected from rats at 30, 60 and 90 days intervals and in the weaned progeny during the experimental period. Feed was withdrawn 12 h before the blood collection and blood was collected through retro-orbital venous plexus through capillary tubes into anticoagulant coated vacutainers with the following procedure.

The animal was held in the left hand with the thumb and index fingers encircling the neck and applying slight pressure so that the veins will fill and the eyeball will protrude slightly. A blunt 20 gauge needle with a fine point was introduced at the medial or lateral canthus of the eye between the orbit and the eyeball. Capillaries were drilled through the conjunctiva by rotating the needle or pipette in the direction of the larynx, since the plexus is located behind the eye at a depth of 4 to 5 mm in the rat. Blood entered the needle or pipette spontaneously and the bleeding was stopped when the pressure on the neck is removed (Benjamin, 1985) [4]. The blood samples collected into the vacutainers were used for estimation of all hematological parameters.

2.3 Haematology

After collection of whole blood samples into pre-treated anticoagulant tubes, the following blood parameters were analyzed as per Benjamin, (1985).

a) Total Erythrocyte Count (TEC)
b) Total Leukocyte Count (TLC)
c) Hemoglobin (Hb) concentration
d) Packed Cell Volume (PCV - Hematocrit)

3. Results and Discussion

3.1 Total Erythrocyte Count (TEC), Total Leukocyte Count (TLC) & Hemoglobin (Hb) concentration

The hemoglobin (g %) levels in all the experimental groups were depicted in table 2 and fig.1. The mean hemoglobin (g%) concentration of group II is significantly (P<0.05) decreased at 30 days (8.25), 60 days (8.35), 90 days (9.15) in rats and also in progeny (9.22) when compared to the control group (11.75, 12.35, 12.17, respectively and 11.15 in progeny). A significant (P<0.05) protective action was observed among group V (8.42, 8.55, 9.45 and 9.35, respectively) and group VI (8.68, 9.35, 9.35 and 9.3, respectively) both in rats and its progeny. A significant (P<0.05) difference was noticed in group III (10.15, 10.50, 10.15 and 10.35, respectively) rats and progeny, group IV rats and its progeny when compared to control (11.15, 11.35, 12.22 and 11.35) group.

The PCV (%) values of all the experimental groups were presented in table 3 and Fig. 2. The mean packed cell volume (%) percentages are significantly (P<0.05) decrease in the toxin group rats at 30 days (13.17), 60 days (15.15), 90 days (25.22) and in its progeny (30.15) when compared to its control group I (43.35, 45.15, 50.22 and 46.25) rats and its progeny respectively. Similarly, a significant (P<0.05) difference was recorded among rats and its progeny in protective groups (group V - 30.25, 25.15, 40.22 and 35.15), group VI- 25.25, 35.22, 32.30 and 33.15) and positive control groups (group III -35.22, 40.15, 45.22 and 35.22, group-IV 40.22, 41.30, 42.22 and 40.150) when compared with control group respectively.

The details of mean TEC (mill/cmm) of all the experimental groups were presented in table 4 and Fig.3. The mean TEC (mill/cmm) was significantly (P<0.05) decreased in the toxin group of adult rats at 30 days (6.97), 60 days (7.27) and 90 days (7.42) compared to control group (8.27, 8.40 and 8.15, respectively). Similarly there was a significant (P<0.05) decrease in the TEC of its progeny in toxin group (7.15) compared to control group (8.15). A significant (P<0.05) protective effect was observed in group V (7.5, 7.5, 7.6 and 7.82 in progeny) and group VI (7.25, 7.57, 7.55 and 8.22) rats and its progeny, respectively. The TEC values in group III (8.35, 8.25, 8.22 and 8.17, respectively) and group IV (8.15, 8.27, 8.25 and 8.20, respectively) were comparable with each other in rats and its progeny.

The results of present study were in agreement with several reports (Costa *et al.,* 1996, Burden *et al.,* 1998 and Adjroud, 2009) [5, 6, 7].

Cr-VI can penetrate rapidly through the membrane of erythrocyte and enter the cell and accumulates in the erythrocytes of exposed workers (Lewalter *et al.,* 1985, Minora and Cavalleri, 1998 and Stridsklev *et al.,* 2004) [8,. 9, 10]. The red blood cell chromium is currently considered as the best indicator of hexavalent chromium exposure as reported by Costa *et al.,* (1996) [5].

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 (Gurer *et al.,* 1998) [11]. This decrease may be due to binding of Cr-VI to beta-chain of haemoglobin in the erythrocyte (Barceloux, 1999) [12] explaining the depleted concentrations of heamoglobin (Adjroud, 2009) [7].

Table 2: Mean values of hemoglobin (g %) as effected by various experimental diets in different groups of rats and its progeny.

Groups	Age in days			
	30 Days	60 Days	90 Days	Progeny
Group I	11.75^{a}±0.62	12.32^{a}±0.13	12.17^{a}±0.08	11.15^{a}±0.06
Group II	8.25^{c}±0.10	8.35^{e}±0.06	9.15^{d}±0.06	9.22^{c}±0.08
Group III	10.15^{b}±0.06	10.5^{c}±0.04	10.15^{b}±0.06	10.35^{b}±0.06
Group IV	11.15^{a}±0.06	11.35^{b}±0.06	12.22^{a}±0.11	11.35^{a}±0.06
Group V	8.425^{c}±0.08	8.55^{e}±0.06	9.42^{c}±0.04	9.35^{c}±0.06
Group VI	8.68^{c}±0.19	9.35^{d}±0.06	9.35^{cd}±0.06	9.3^{c}±0.07

S.E – Standard Error

Means bearing common superscripts do not differ significantly (P<0.05)

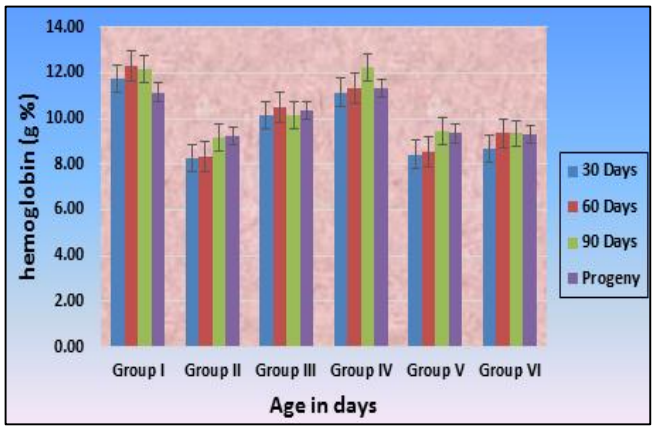

Fig 1: Mean values of hemoglobin (g %) as effected by various experimental diets in different groups of rats and its progeny

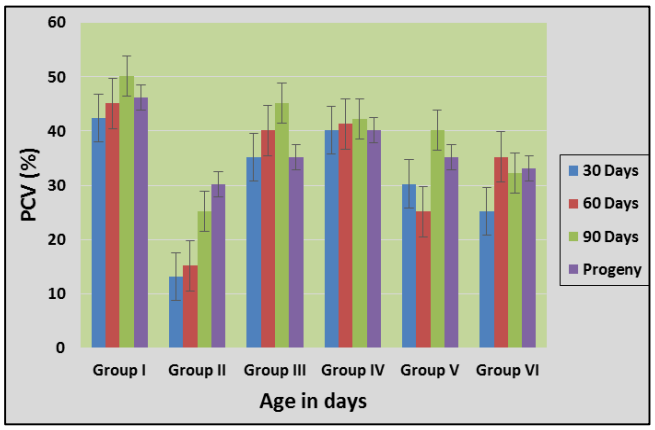

Fig 2: Mean values of PCV (%) as effected by various experimental diets in different groups of rats and its progeny

Table 3: Mean values of PCV (%) as effected by various experimental diets in different groups of rats and its progeny

Groups	Age in days			
	30 Days	60 Days	90 Days	Progeny
Group I	42.35^{a}±0.06	45.15^{a}±0.06	50.22^{a}±0.08	46.25^{a}±0.10
Group II	13.17^{f}±0.08	15.15^{f}±0.06	25.22^{f}±0.08	30.15^{e}±0.06
Group III	35.22^{c}±0.08	40.15^{c}±0.06	45.22^{b}±0.11	35.22^{c}±0.08
Group IV	40.22^{b}±0.08	41.3^{b}±0.07	42.22^{c}±0.11	40.15^{b}±0.06
Group V	30.25^{d}±0.10	25.15^{e}±0.06	40.22^{d}±0.08	35.15^{c}±0.06
Group VI	25.22^{e}±0.08	35.22^{d}±0.08	32.3^{e}±0.10	33.15^{d}±0.06

S.E – Standard Error

Means bearing common superscripts do not differ significantly (P<0.05)

Table 4: Mean values of TEC (mill/cumm) as effected by various experimental diets in different groups of rats and its progeny.

Groups	Age in days			
	30 Days	60 Days	90 Days	Progeny
Group I	8.27^{a}±0.04	8.4^{a}±0.04	8.15^{a}±0.06	8.15^{a}±0.06
Group II	6.97^{c}±0.13	7.3^{c}±0.04	7.42^{c}±0.08	7.15^{c}±0.06
Group III	8.35^{a}±0.06	8.25^{a}±0.02	8.22^{a}±0.08	8.17^{a}±0.08
Group IV	8.15^{a}±0.06	8.3^{a}±0.04	8.25^{a}±0.06	8.2^{a}±0.08
Group V	7.5^{b}±0.04	7.5^{b}±0.09	7.65^{b}±0.06	7.85^{b}±0.17
Group VI	7.35^{b}±0.06	7.5^{b}±0.13	7.55^{bc}±0.06	8.22^{a}±0.04

S.E – Standard Error

Means bearing common superscripts do not differ significantly (P<0.05)

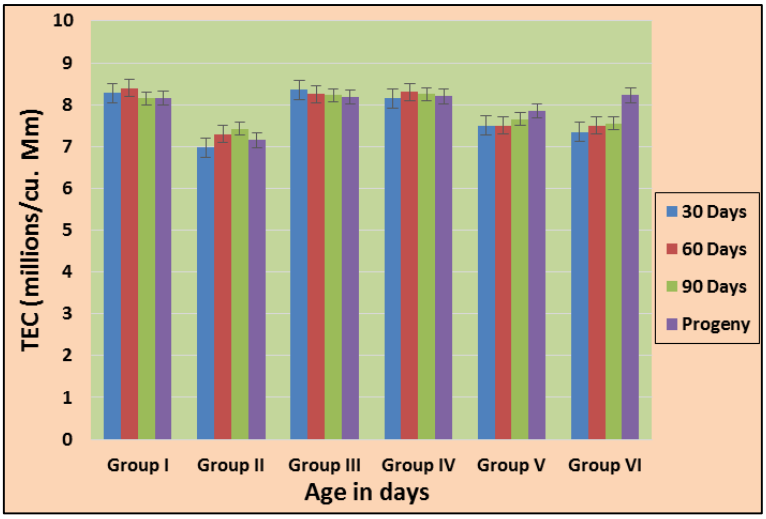

Fig 3: Mean values of TEC (mill/cumm) as effected by various experimental diets in different groups of rats and its progeny.

3.2 Total leukocyte count (TLC-Thousands/cmm)

The mean values of TLC (thousands/cmm) of all experimental groups were presented in table 5 and Fig. 4. The mean total leucocytes count (thousands/cmm) in the group II is a significantly (P<0.05) decreased during 30 (3.94), 60 (3.91), 90 days (3.76) in rats and its progeny (3.86) when compared to control group (10.52, 10.42, 12.25 and 10.35, respectively). A significant protective effect was observed in group V (7.38, 7.42, 7.65 respectively and 7.55 in the progeny) and group VI (6.53, 6.45, 6.55 respectively and 6.42 in the progeny) when compared with the control group. There was a significant difference in group III (9.65, 12.20, 10.45 and 11.32 respectively in rats and its progeny) and group IV (8.53, 8.40, 8.35 and 8.42 respectively in rats and its progeny) when compared to control group.

These findings are in co-occurrence with the findings of the Geetha *et al*., (2005) [14] and Adjroud, (2009) [7]. The reduction in TLC 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. In 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 (Geetha *et al*., 2005) [14].

One major lesion associated with Cr-VI toxicity is DNA damage in the intact lymphocytes (Costa *et al*., 1996) [5]. Similarly there will be a dose dependent increase in DNA strand breakage in the rat peripheral lymphocytes upon exposure to Cr-VI toxicity (Gao *et al*., 1992) [15].

The adverse effects of Cr-VI were significantly decreased in vitamin C and *Emblica officinalis* groups because of its ameliorative/ protective role. As the vitamin C acts as a biological antioxidant by donating an electron to free radical species, there by intercepting the radical chain reaction in biological membranes and protects from the noxious effects like decrease TLC counts in rats (Kesinger and Stevens, 2009) [13].

Table 5: Mean values of TLC (thousands/cumm) as effected by various experimental diets in different groups of rats and its progeny

Groups	Age in days			
	30 Days	60 Days	90 Days	Progeny
Group I	10.52^{a}±0.2	10.42^{b}±0.08	12.25^{a}±0.10	10.35^{b}±0.06
Group II	3.94^{f}±0.020	3.91^{f}±0.006	3.76^{f}±0.08	3.86^{f}±0.03
Group III	9.65^{b}±0.06	12.2^{a}±0.09	10.45^{b}±0.15	11.32^{a}±0.13
Group IV	8.53^{c}±0.13	8.4^{c}±0.10	8.35^{c}±0.06	8.42^{c}±0.08
Group V	7.38^{d}±0.04	7.42^{d}±0.08	7.65^{d}±0.06	7.55^{d}±0.06
Group VI	6.53^{e}±0.03	6.45^{e}±0.06	6.55^{e}±0.06	6.42^{e}±0.08

S.E – Standard Error

Means bearing common superscripts do not differ significantly (P<0.05)

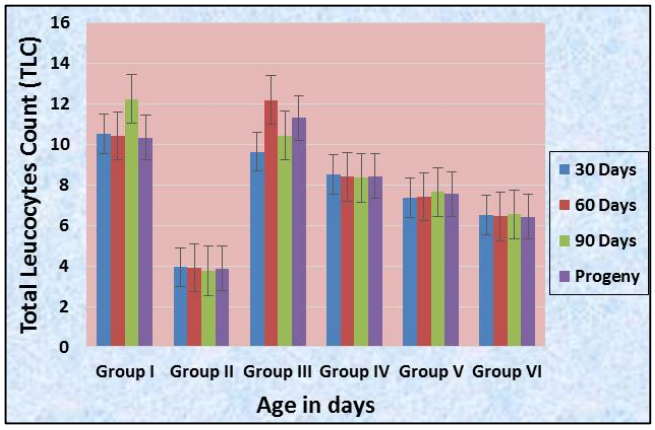

Fig 4: Mean values of TLC (thousands/ cumm) as effected by various experimental diets in different groups of rats and its progeny

4. Conclusion

From the present study it may be concluded that the haematological profile showed a significant decrease in the overall mean values of Hb, TEC, PCV and TLC at significant (p<0.05) level in comparison with the other groups (I,III,IV,V and VI). Hence it can be concluded that the progressive toxico-pathological effect of hexavalent chromium @500 ppm in the form of potassium dichromate ($K_2Cr_2O_7$) can be moderately reduced by supplying vitamin C @100mg/kg body weight orally in drinking water and by feeding emblica officinalis orally @2% in feed are equally protective and ameliorative in counteracting the adverse effects of hexavalent chromium toxicity @500 ppm in the form of potassium dichromate in female wistar rats.

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