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Chromium load in hexavalent chromium toxicity in wistar rat

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Abstract
Hexavalent chromium salts are recognized as occupational health hazard for more than 160 years. Chromium (Cr – VI) is generally considered 1000 times more toxic than (Cr –III). An experiment was conducted with six dietary groups of female wistar rats. Group I, III and IV are control, vitamin C control and emblica officinalis control groups respectively and group V and VI are ameliorative groups. Group II was provided with potassium dichromate 500ppm in drinking water orally for three months along with basal diet. The final result has been interpreted as there is a significant increase in chromium load in toxin group rats. While group V and VI showed mild to moderate improvement when compared to toxin group. Control group values are apparently normal. From the results of this study it has been concluded the presence of chromium in the water at the rate of 500ppm resulted in significant toxicity and this toxic effect was reduced by protective agent vitamin C and ameliorative agent emblica officinalis.

Keywords: Chromium load, female wistar rats, hexavalent chromium toxicity

Introduction
Chromium is a naturally occurring element found in animals, plants, rocks, soil, volcanic dust and gasses (Nejla Soudani *et al*., 2010) [1]. Chromium (Cr – VI) is a primary contaminant due to its toxicity to humans, animals, plants and micro organisms. Chromium has been studied since the end of 19th century, when carcinogenic effect of hexavalent chromium were discovered (Pechova and Pavlata, 2007) [2]. Selected chromium compounds, particularly hexavalent ones are carcinogens, corrosives, delayed contact sensitizers and have the kidneys as their primary target organ (Gad Shayne, 1989) [3].

Materials and Methods
Experimental Animals
Experimental was conducted for a period of four months with a total of 120 weaned female wistar rats (*Rattus norvegicus*) weighing between 90-150g were obtained from Sanzyme limited, Gaganpahad, Hyderabad. Animals were housed in polypropylene cages in an air-conditioned animal house with 12h-12h light-dark cycles. Rats in all the replicate groups were reared under uniform standard conditions throughout the experiment. These rats were maintained under standard managemental conditions with the provision of feed a standard laboratory diet twice daily and water *ad libitum* throughout the experiment.
The rats were quarantined and acclimatized for a period of 2 weeks was observed before the start of experiment. These experimental animals were identified by different color markings on their foreheads and body parts with picric acid. Experiment was conducted according to the guidelines of Institutional Animal Ethics Committee (*No I/4/14, date 27.11.2014*).

Experimental Design
After an acclimatization period of 2 weeks the 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).

The Department of Veterinary Pharmacology & Toxicology with the following treatment schedule

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 3 months+*Emblica officinalis* powder given @ 2% in feed for 3 months.

Experimental Feed

All the weaned wistar rats were fed with rat feed in the form of pellets (*NIN feed standard*) was procured from National Institute of Nutrition (NIN), ICMR, Hyderabad. The six (6) groups of rats were fed with the diet (at random) and sanitized drinking water *ad libitum*.

Chromium load

Homogenates tissues were digested with acid mixture containing nitric acid, H_2SO_4 and perchloric acid in a ratio of 6:1:1 a regulated heater. After digestion the acid mixture was evaporated with occasional additions of triple distilled water and the solution thus obtained was used for chromium estimation by atomic absorption spectrophotometer and measured in parts per million.

Results

Chromium load (ppm) in uterus

The details of mean values of chromium load (ppm) at the end of the experimental study were detailed in table 1 and figure 1. chromium load is significantly (P<0.05) increased means of chromium load(ppm) in the uterus and fallopian tubes in the toxin group at 90 days (40.22) interval of experiment when compared to control group (36.52). Contrary to this a significant (P<0.05) decrease levels of chromium load at 30 days (25.63) and 60 days (21.44) were observed when compared to control group (33.65 and 75.96). There is a significant (P<0.05) protective effect was noticed among V group (161.56, 20.16 and 26.45) and group VI (45.96, 32.55 and 26.10) during 30, 60 and 90 days of experiment. There is a significant (P<0.05) difference of means were observed in group III (28.75, 25.37 and 40.76) and in group IV (21.96, 26.74 and 28.22).

Table 1: Mean values of chromium load in uterus (ppm) as effected by various experimental diets in different group of rats.

Groups	Age in days		
	30 Days	60 Days	90 Days
Group I	33.65^{c}±0.02	75.96^{a}±0.005	36.52^{b}±0.01
Group II	25.63^{e}±0.04	21.44^{e}±0.005	40.22^{a}±0.01
Group III	28.75^{d}±0.008	25.37^{d}±0.005	40.76^{a}±0.005
Group IV	21.96^{f}±0.01	26.74^{c}±0.01	28.22^{c}±0.01
Group V	161.56^{a}±0.17	20.16^{f}±0.008	26.45^{c}±0.01
Group VI	45.96^{b}±0.005	32.55^{b}±0.01	26.10^{c}±2.66

S.E – Standard Error

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

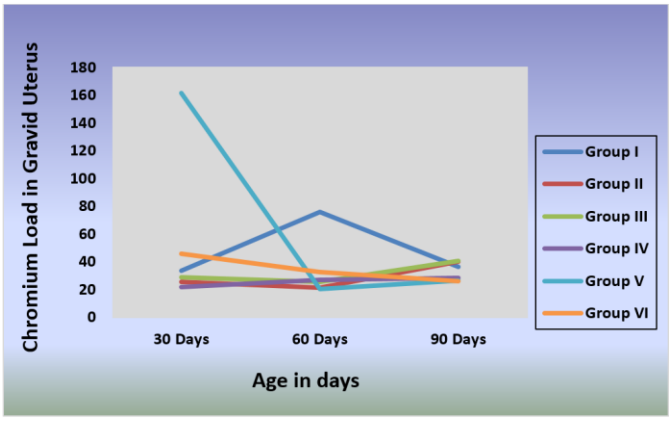

Fig 1: Mean values of chromium load in uterus (ppm) as effected by various experimental diets in different group of rats.

Chromium load (ppm) in ovaries of rats and dams after weaning of progeny

The chromium load (ppm) levels of all experimental groups were depicted in table 2 and figure 2. There is a significant (P<0.05) increase in the chromium load (ppm) in the ovary of rats and dams (after weaning of progeny, approximately 150 days) in the toxin group at 30 days (22.66), 60 days (27.04) and 90 days (27.28) and (28.04) when compared to control group rats (3.43, 3.25, 3.26 and 3.26) respectively. There is a significant protective action against chromium was observed in group V (17.43, 18.40, 19.60 and 18.60) and group VI (15.02, 16.05, 18.36 and 18.36) respectively with vitamin C and *E. officinalis*, similarly there is a significant (P<0.05) difference in chromium load in group III (3.06, 3.15, 3.17 and 3.12) and in group IV (3.30, 0.15, 3.17 and 3.14) was also documented.

Table 2: Mean values of chromium load in ovary (ppm) as effected by various experimental diets in different group of rats.

Groups	Age in days			
	30 Days	60 Days	90 Days	GEST
Group I	3.43^{d}±0.08	3.25^{d}±0.008	3.26^{d}±0.01	3.26^{c}±0.01
Group II	22.66^{a}±0.018	27.04^{a}±0.02	27.28^{a}±0.008	28.04^{a}±0.01
Group III	3.06^{e}±0.02	3.15^{e}±0.017	3.176^{d}±0.008	3.12^{c}±0.008
Group IV	3.3^{d}±0.05	0.15^{f}±0.005	3.17^{d}±0.008	3.14^{c}±0.005
Group V	17.43^{b}±0.08	18.4^{b}±0.05	19.6^{b}±0.05	18.6^{b}±0.05
Group VI	15.02^{c}±0.008	16.05^{c}±0.02	18.36^{c}±0.18	18.36^{b}±0.18

S.E – Standard Error

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

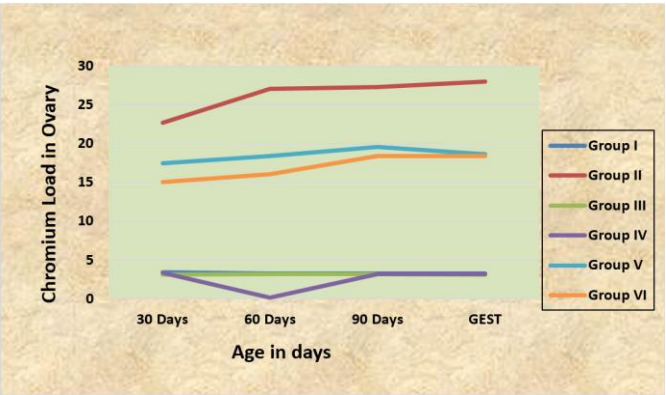

Fig 2: Mean values of chromium load in ovary (ppm) as effected by various experimental diets in different group of rats.

Discussion

Chromium load in uterus (ppm)

A significant increase in the chromium load in the uterus and fallopian tubes in the toxin group at 90 days of experiment when compared to control group. However, there is a significant decrease in chromium load at 30 days and 60 days were recorded when compared to control group during respective days. Where as in groups III and IV a significant difference was observed in comparison to control group. These results were in agreement with the findings of Kanojia *et al.*, (1996) [4] and Snejana petrovici *et al.*, (2008) [5].

The increase in chromium load in the uterus with fallopian tubes may be due to significant increase in the chromium levels in blood, placenta and fetuses when compared to control group of rats. This is in agreement with the findings of Kanojia *et al.*, 1996 [4]. This higher levels of chromium VI in tissues, which reflects the greater ability of chromium VI to cross the plasma membrane and bind to the intra cellular protein in various tissues which explains greater degree of toxicity of chromium (VI) (Coogan *et al.*, 1991) [6].

The chromium load in the uterus with fallopian tubes is comparatively minimum in groups V and VI by supplementing vitamin C and *E.officinalis* because of its ameliorative role, vitamin C, a biological antioxidant in its nature donates an electron to free radical species, there by interrupting the radical chain reaction in biological membranes and protects animals from increased chromium load.

Chromium loading ovaries (ppm) of rats and dams after weaning of progeny

There is a significant increase in the chromium load in the ovary in the toxin group rats at 30 days ,60 days ,90 days and in the ovaries of dams after weaning of progeny rats when compared to control group rats. There is a significant protective effect in group V and in group VI was noticed in present study. Similarly there is a significant difference in group III and in group IV was also observed when compared to control group. These results were in agreement with the findings of Kanojia *et al.*, (1996) [4] and Snejanapetrovici *et al.*, (2008) [5].

Similarly significant increase in the chromium level in the ovaries of dams were also recorded in this experiment. This is in accordance with the observation of Kanojia *et al*., 1996 [4], who found a significant increase in the chromium levels in blood, placenta and fetuses when compared to control group of rats. This increase may be due to its exposure to hexavalent chromium during its pre-gestation period leading to a significant increase in the chromium levels in blood, placenta and fetuses when compared to control group of rats. This higher levels of chromium VI in tissues, which reflects the greater ability of chromium VI to cross the plasma membrane and bind to the intra cellular protein in various tissues which explains greater degree of toxicity of chromium (VI) by Coogan *et al*.,(1991) [6].

A significant (P<0.05) increase in the chromium level in the ovaries of dams is reduced by supplementing vitamin C and *E. officinalis* because of its ameliorative role. Vitamin C acts as a biological antioxidant by donating an electron to free radical species, there by interrupting the radical chain reaction in biological membranes and protects from the adverse effects like increased chromium load in animals in chromium (VI) toxicity.

From the present study it may be concluded that the hexavalent chromiumis highly toxic on a parameter like chromium load and it was optimum to damage the cellular functions. And these adverse effects were significantly ameliorated by supplementation of vitamin C and emblica officinalis.

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