Detoxification and Toxicity Effects of Hexavalent Chromium in Rats: Evaluation of Early Biomarker

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Abstract—*Background information and objectives: Hexavalent chromium [(Cr (VI)] is highly toxic and a known human carcinogen and its detoxification may further lead to the initiation of toxic effects. To look further insight into the matter, we have conducted this study with respect to dose and duration of exposure to Cr (VI). Methodology: Adult female albino rats were divided in four groups containing six animals each. Cr (VI) compound (K_2 Cr_2 O_7) was administered to experimental groups (Group II, III and IV) through drinking water for a period of 3 months at a dose of 1, 5, and 25 mg/kgbw. Control group (Group I) received RO water only. The animals were kept under observation and their body weight was recorded. Urine samples were collected daily for 1 week after 45 days of exposure and analysed by ion chromatography. The experiment was further continued for 3 months and then sacrificed. Blood (plasma and RBC), liver and kidney were used for the estimation of total chromium (TCr) by atomic absorption spectrometer (AAS). Results and discussion: A gradual weight loss was observed in all groups. But it was not statistically significant when any experimental group was compared with control. Higher doses of Cr (VI) appeared to cause loss of appetite. Toxicity symptoms were more pronounced in high dose group (25 mg/kg). Urinary Cr (measured as TCr) and the end-metabolized product of Cr (VI) was elevated and a dose-response relationship was observed. Trace amount of Cr (VI) in urine of high dose group (25mg/kg) was also detected. Bioaccumulation of TCr in liver was about more than 4 times higher in high dose group (25 mg/kg) when compared to control (2.096 ± 0.285 vs. 0.492 ± 0.192 mg/kg). No considerable accumulation was seen in other exposed group (1 and 5 mg/kg).*
Higher levels of TCr (about 20- fold over control group) were detected in kidney in group of animals exposed to 25mg/kg. Cr content as TCr in RBC was only detected in high dose group (25mg/kg). Conclusions: Pronounced toxic effects of Cr (VI) exposure were observed in high dose group. Higher level of Cr in RBC may reflect excessive exposure to Cr (VI). Elevated levels of TCr in urine may be used as biomarker of Cr exposure.

Keywords*: Hexavalent chromium, trivalent chromium, Cr (VI), Cr (III), ionchromatography*

1. INTRODUCTION

Chromium (Cr) is an important industrial metal and two common form of it [trivalent chromium, Cr (III) and hexavalent chromium, Cr (VI)] are widely used in many industries [1-2]. Cr (VI) is highly toxic and a known human carcinogen by inhalation route, however, a recent study [3] shows carcinogenic potential of Cr (VI) by oral route as well. On the other hand, Cr (III) is known to act as a micronutrient but toxic at higher concentration. In oral exposure, the parent compound (Cr VI) is mainly detoxified to Cr (III) by saliva and stomach by way of reduction [4] and a small amount may escape un-reduced that is further distributed to various organs, tissues and blood and then finally eliminated in urine in the form of total Cr (TCr). During this process free radicals and Cr- intermediates [(Cr (IV) and Cr (V)] are generated that are known to cause oxidative stress and DNA damage [5] resulting in further initiation of chromium toxicity. Study reported by OSHA [6] has failed to detect Cr (VI) in urine as Cr (VI).

So far, little is known about the distribution and metabolism of Cr (VI) in the different tissues and its elimination in urine as TCr. To look further insight into the matter, we have conducted this study to know the detoxification, distribution and elimination of Cr (VI) with respect to dose and duration of exposure to Cr (VI).

2. METHODOLOGY

This study was conducted in adult female albino rats (average weight 238 ±10.63 g). They were divided in 4 groups containing 6 animals each. Hexavalent chromium compound (K_2 Cr_2 O_7) was administered to experimental groups (Group

II, III and IV) through drinking water for a period of 3 months at a dose of 1, 5, and 25 mg/kgbw corresponding to about 1/50th, 1/10th and 1/2 of LD 50 dose. Control group (Group I) received RO water only. Animals were kept under observation and their body weight was recorded bi-weekly. Sign and symptoms were also recorded. Urine samples were collected daily for 1 week after 45 days of exposure. Samples were filtered through 0.45 µm Acrodisc® syringe filter and suitably diluted to analyze Cr (III) and Cr (VI) by ionchromatography employing USEPA method [7]. The experiment was further continued for a period of 3 months and the animals were sacrificed. Blood was collected by cardiac puncture and plasma and RBC were separated; tissues (liver and kidney) were also removed and washed with normal saline. All the samples were preserved at -20^{0}C till further analysis of total chromium by graphite furnace atomic absorption spectrophotometer (GF AAS).

3. RESULTS AND DISCUSSION

Table 1 summaries the effects of dose and duration of exposure of Cr (VI) on the body weight of animals. A gradual weight loss was observed in all groups. But it was not statistically significant when any experimental group was compared with control. Higher doses of Cr (VI) appeared to cause loss of appetite. Urinary elevated levels of Cr (III) as the end-metabolized product of Cr (VI) were observed more pronounced in exposed groups when compared to control group, a dose-response relationship was observed (Table 2). Trace amount of Cr (VI) in urine of high dose group (25mg/kgbw) was also detected (Table 2 & Fig. 1). However, studies reported so far failed to detect Cr (VI) in urine. Fig. 2 shows the standard chromatogram of Cr (III) and Cr (VI). Distribution and accumulation pattern of Cr (VI) (measured as total Cr) in RBC and other tissues are shown in Table 3. As indicated in the table, the level of TCr in kidney of high dose group (25mg/kg) was about 20-fold high over control group (6.9 ±.3.146 *vs* 0.353 ± 0.123 mg/kg). It was still higher than the level detected in liver (2.906 ± 0.285 mg/kg). Cr (VI) is known to enter into RBC where it is reduced to Cr (III). The elevated level of Cr (III) measured as TCr (14.14±11.41, µg/g) in this study was only registered in high dose group (Table 3).

Table 1: Effects of Cr (VI) on body weight of female rats with respect to duration and dose of exposure

Expo -sure	Group I	Group II	Group III	Group IV
Days/ Dose	Control 0 mg/kg	Cr-exposed 1mg/kg	Cr-exposed 5mg/kg	Cr-exposed 25mg/kg
0	224.8 ± 16.6	247.5 ± 30.5	238.6 ± 19.3	246.8 ± 24.8
7	223.3 ± 19.4	247.3 ± 31.4	231.8 ± 16.9	244.3 ± 20.1
14	225.3 ± 19.9	246.8 ± 32.3	234.2 ± 20.4	245.0 ± 21.9
21	228.1 ± 22.9	246.6 ± 30.8	232.8 ± 15.9	243.0 ± 20.9
28	228.3 ± 19.7	245.1 ± 37.9	231.5 ± 16.2	242.5 ± 20.5
36	221.6 ± 18.6	243.1 ± 34.9	230.5 ± 13.9	242.6 ± 18.8
45	219.6 ± 21.4	235.6 ± 35.8	217.3 ± 14.3	232.5 ± 21.4

Table 2: Levels of urinary chromium in control and Cr-exposed rats after 45 days of exposure

S. No.	Treatment ($K_2Cr_2O_7$) mg/kgbw	Cr (III) (ppb) mg/kgbw	Cr (VI) (ppb) mg/kgbw
1	Control	N.D	ND
2	1	14.13	ND
3	5	43.25	0.98
4	25	106.51	1.42

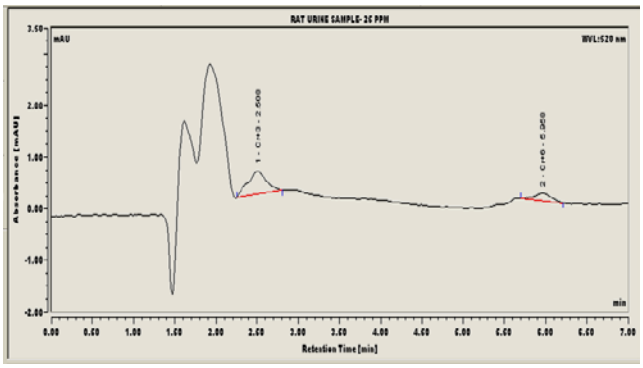

Fig. 1: Chromatogram showing elution profile of Cr (III) and Cr (VI) in urine sample of high dose group

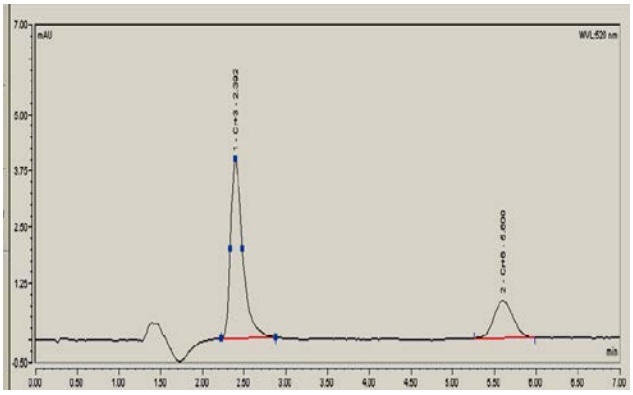

Fig. 2: Standard chromatogram of Cr (III) and Cr (VI) (300 ppb and 5ppb was applied respectively)

Table 3: Distribution of Cr (measured as TCr) in liver, kidney and RBC of control and Cr- exposed group

Tissue	Exposure (3 months)			
	Liver (µg/g)			
	Control	**1 mg/kg bw**	**5 mg/kg bw**	**25 mg/kg bw**
A	0.285	0.587	0.646	2.35
B	0.218	0.510	0.435	3.16
C	0.712	0.332	0.336	3.03
D	0.492	0.441	0.284	3.02
E	0.521	0.448	0.368	ND
F	0.726	0.210	0.417	2.902
Mean ± SD	**0.492 ± 0.192**	**0.421 ± 0.121**	**0.414 ± 0.115**	**2.906 ± 0.285**
Kidney (µg/g)				
A	0.530	0.376	0.590	9.624
B	0.496	0.20	1.250	1.022
C	0.295	0.219	0.643	8.67
D	0.188	0.288	0.425	6.35
E	0.346	0.160	0.461	ND
F	0.259	0.22	1.100	8.926
Mean ± SD	**0.353 ± 0.123**	**0.243 ± 0.07**	**0.744 ±0.315**	**6.918 ± 3.146**
RBCs (µg/g)				
A	ND	ND	ND	14.94
B	ND	ND	ND	4.94
C	ND	ND	ND	33.19
D	ND	ND	ND	20.39
E	ND	ND	ND	ND
F	ND	ND	ND	10.25
Mean ± SD				**14.141 ± 11.406**

4. CONCLUSIONS

This study may suggest that estimation of Cr in RBC as TCr is an indicator to excessive exposure to Cr (VI) and it may be regarded as biomarker of Cr (VI) exposure. Elevated levels of Cr in urine measured as TCr may also serve as biomarker of Cr exposure but it cannot differentiate the kind of exposure to Cr – species (Cr III or Cr VI).

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REFERENCES

[1] JO Nriagu and JM Pacyna (1988). Quantitative assessment of worldwide contamination of air, water and soil by trace metal Nature 33, 134-139.

[2] ASTDR (2002). ASTDR Tox- Profile of Chromium, U.S. Department of health and Human Services, Agency for Toxic Substance and Disease Registry, U.S.A.

[3] A Linos, P Athanassios, CA Christophi, et al (2011). Oral ingestion of hexavalent chromium through drinking water and cancer mortality in an industrial area of Greece -an ecological study Env. Health: A global access Science Source, 10 (suppl.) 1: 50.

[4] De Flora S , Camoirano A , Bagnnasco M, Bennicelli C, E Corbett G, and Brent D Kerger (1997), Estimates of the chromium(VI) reducing capacity in human body compartments as a mechanism for attenuating its potential toxicity and carcinogenicity Carcinogenesis, 18(3),531-537.

[5] Wang Xiao-Feng, Xing Ming-Laun, Shan Ying et al. (2006). Oral administration of Cr (VI) induced oxidative stress, DNA damage and apoptotic cell death in mice doi.101016/I tox2006.08.005

[6] OSHA (2006) Occupational exposure to hexavalent chromium-71:1009-10385 (https://www.osha.gov/pls/oshaweb/owadisp.show_document)

[7] [7] US EPA (2011) Method 218.7 Determination of dissolved hexavalent chromium in drinking water, groundwater and industrial wastewater effluents by Ion Chromatography, Environmental Protection Agency, and Cincinnati, Ohio 45268.(http://water.epa.gov/drink)