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PATHOPHYSIOLOGICAL EFFECT OF CHROMIUM - VI ON SOME HAEMOCHEMICAL PARAMETERS IN A MAMMALIAN MODEL

Indrani Chakraborty[1], Rafi Ahammed Reja[2], Sougata Karmakar[2] and Dr. Nirmal Pradhan[2]*

[1]Sister Nivedita Girl's College, Kolkata
[2]PG Dept. of Physiology, Hooghly Mohsin College, Hooghly.

Corresponding Author: Dr. Nirmal Pradhan
PG Dept. of Physiology, Hooghly Mohsin College, Hooghly.

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ABSTRACT

The existence of Chromium(Cr) was discovered by Vauquelin in 1797 and its physical and chemical properties have sequentially been determined afterwards. Most of the studies on health effects involve exposure to chromium 0, III, IV and chromium-VI compounds. Cr-VI in the environment is almost always related to anthropogenic activity. The most common form of Cr-VI is readily solubilised from most soil and transported through water that contacts the soils and used for industrial and commercial productions which have become more prevalent environmental contaminants. Accidental or intentional ingestion of extremely high doses of Cr-VI compounds has resulted in severe respiratory, cardiovascular, gastrointestinal, hematological, hepatic, renal and neurological effects in man as a part of the sequelae leading to death or in patients who survived because of medical treatment. But no reports are available on chronic oral exposure of Cr-VI at equivalant to human RDA doses on hematological parameters in rats as the mammalian model. In the present study, Cr-VI has been administered orally through gavage to adult male albino rats at the doses of 1mg, 1.5mg and 2mg /100gm body weight/day, accordingly to Gr. 1, 2 and 3 for consicutive 33 days with water *ad libitum.* The results showed no significant changes in body weight gain, Haemoglobin level, blood glucose level, serum protein, serum alkaline phosphatase activity and SGPT activity. Only the mild increased activity was found in SGOT in group-3. So, it can be surmised that, Cr-VI has no such potent toxic effects in this treated doses and duration on male rats as the mammalian system.

KEYWORDS: ALKP, Blood Sugar, Chromium-VI, Haemoglobin, SGOT, SGPT.

INTRODUCTION

The existence of chromium was discovered by Vauquelin in 1797 of which physical and chemical properties have sequentially been determined afterwards.[1] Cr-III occurs naturally in soil and mineral deposits, while Cr-VI encountered in natural unpolluted soils. Cr-III is relatively immobile in soil or sediments. The most common form of Cr-VI is readily solubilised from most soil and transported through water that contacts the soils. As Cr-chemicals are widely distributed and used in both developing and industrialized nations for industrials and commercial productions. Chromium and more particularly Cr-VI, have become prevalent environmental contaminants.[2] Both humans and animals are capable of converting inactive inorganic chromium(III) compounds to physiologically active forms. Although chromium (III) has been reported to be an essential nutrient, exposure to high levels via inhalation, ingestion or dermal contact may cause some adverse health effects. Most of the studies on health effects involve exposure to chromium 0, III, IV and chromium VI compounds, in addition, chromium IV was used in an inhalation study to determine permissible exposure levels for workers involved in producing magnetic tape.[3] Chromium III occurs naturally in the environment and chromium VI in the environment is almost always related to anthropogenic activity and has been reported as an industrial toxin.[4]

Accidental or intentional ingestion of extremely high doses of chromiumVI compounds by humans has resulted in severe respiratory, cardiovascular, gastrointestinal, hematological, hepatic, renal and neurological effects as part of the sequelae leading to death or in patients who survived because of medical treatment.[4,5] But no reports are available on chronic oral exposure of Cr-VI at equivalant to human RDA doses on hematological parameters in rats as the mammalian model. So, in the present work, the effort have been given to study the changes of vital haemochemical parameters due to chronic oral administration of Cr-VI through gavage at equivalant to human RDA doses in adult male albino rats.

MATERIALS AND METHODS

A) Animal grouping and maintenance

Experiments were carried out with adult male albino rats weighing 120-130g. They were kept into 4 groups having 5 animals each as follows –

GROUP	DOSE	DURATION
Expt. Gr-1	1mg/100gm body wt./day	33 Days
Expt. Gr-II	1.5mg/100gm body wt./day	33 Days
Expt. Gr-III	2mg/100gm body wt./day	33 Days
Expt. Gr-IV	Vehicle Control	33 Days

All animals were pair-fed and the composition of diet was same (as available standard rat diet)[6] Water was given *ad libitum*. Animals were maitained under standard laboratory conditions (temp. 25 ± 2°C, 12/12 hr. dark and light, relative humidity 40 - 60 %). Animal experiments were performed accordig to the ethical guidelines under IAEC, The University of Burdwan, Burdwan, West Bengal, India.

B) Drug preperation and application

Chromium VI salt was prepared as - potassium chromate (K_2CrO_4) 1 gm was dissolved in 1litre of distilled water, so, 1 ml of solution contains 1mg of potassium chromate. Cr VI solution was supplemented by force feeding (oral root).[7]

C) Collection of Blood and serum preperation

At the end of 33 days, blood from rats of different groups were taken by syringe directly through Cardiac puncture. All sampling were performed between 1 pm to 3 pm in order to avoid diurnal variation on the parameter observed in the study.[8] The bottle in which blood was collected for serum preparation was kept tilted for 15 min and then serum was collected in separate eppendorf tubes (2.5ml) by decanting carefully from the bottle. Serum from each eppendorf tube was then centrifused at 1500 rpm for 3 min and then the fresh serum was transferred to new eppendorf tubes (2.5ml).

D) Measurement of Different Parameters

The body weight gain was calculated on the basis of body growth rate % at the day of sacrifice.

Estimation of Haemoglobin (gm%) was done by Sahli's Haemoglobinometer.[9] The total Serum protein was measured by Lowry et. al, 1951.[10] By standard kits, Total Blood Glucose Level, Serum Alkaline Phosphatase (ALKP)[11] Serum Glutamate Oxaloacetate Transaminase (SGOT) and Serum Pyruvate Oxaloacetate Transaminase (SGPT) activities were measured.[12]

E) Statistical Calculations

Data were expressed as mean ± SD. Statistical significance was determined using Student's t test. SPSS-10 software was used for statistical analysis. Differences were considered significant if $P<0.1$.

RESULTS

In the present study, the Cromium VI was administered to adult male rats through oral gavage at the doses i.e. 1mg,1.5mg and 2mg/100gm body weight/day accordingly to different groups for consicutive 33 days. The results showed that Chromium supplementation has no significant effect upon body weight gain, haemoglobin (gm%), blood glucose level, serum protein level, serum alkaline phosphatase activity and in SGPT activity but mild rise in SGOT activity was found in group 3 (2 mg) when compared with vehicle treated control.

A) Changes in Body Weight Gain

Table 1: Results showing the changes in body weight gain in different groups, treated with 1mg, 1.5mg and 2mg potassium chromate /100gm body weight/day, accordingly (GR 1-3) for consicutive 33 days. No significant change ($P>0.1$) was found when compared with control group. Values are expressed as means ± SD, N=5.

Group	Average Body wt. gain (gm%) ± SD	P Values (Comparing with control)
Expt. GR- 1	16.25 ± 3.04	P>0.1
Expt. GR- 2	15.81± 2.63	P>0.1
Expt. GR- 3	15.34 ± 2.01	P>0.1
Control GR	16.84 ± 2.19	

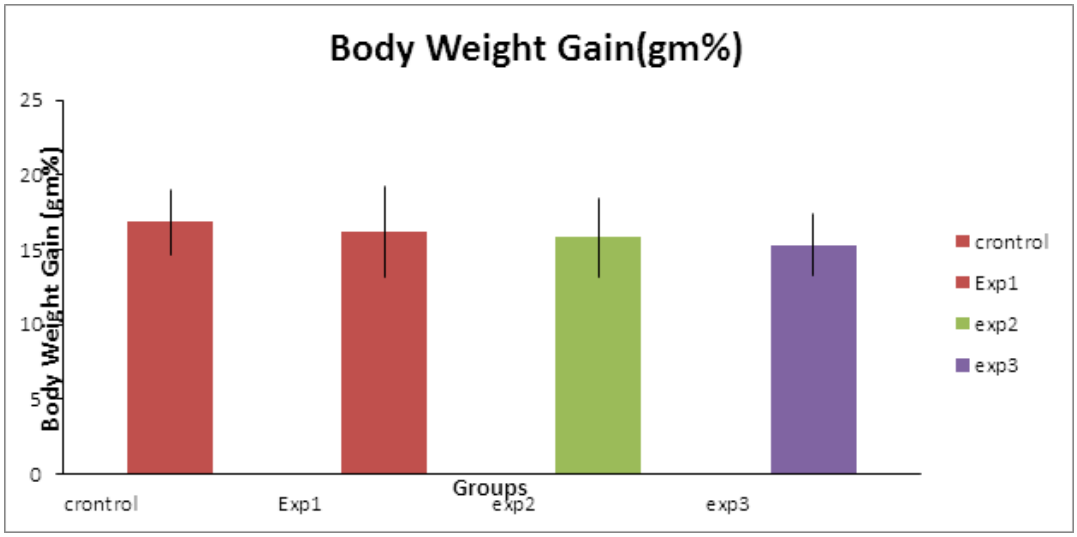

Figure 1: Graphical Representation of Relative changes of body weight gain(gm%) in different groups.

B) Changes in Blood Haemoglobin

Table 2: Results showing the changes in haemoglobin in different groups, treated with 1mg, 1.5mg and 2mg potassium chromate /100gm body weight/day, accordingly (GR 1-3) for consicutive 33 days. No significant change (P>0.1) was found when compared with control group. Values are expressed as means ± SD, N=5.

Group	Blood Hb (g/100ml) ± SD	P Values (Comparing with control)
Expt. GR- 1	12.30 ± 1.56	P>0.1
Expt. GR- 2	12.3 ± 0.14	P>0.1
Expt. GR- 3	12.07 ± 1.06	P>0.1
Control GR	12.20 ± 1.12	

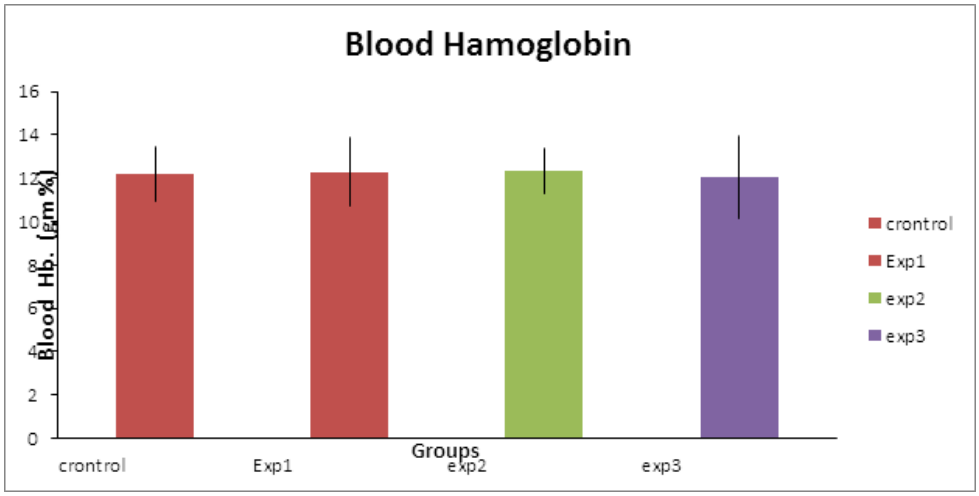

Figure 2: Graphical representation of relative changes of haemoglobin in different groups.

C) Changes in Serum Protein

Table 3: Results showing the changes in Serum Protein in different groups, treated with 1mg, 1.5mg and 2mg potassium chromate /100gm body weight/day, accordingly (GR 1-3) for consicutive 33 days. No significant change (P>0.1) was found when compared with control group. Values are expressed as means ± SD, N=5.

Group	Serum Protein (g/100ml) ± SD	P Values (Comparing with control)
Expt. GR- 1	7.0 ± 0.57	P>0.1
Expt. GR- 2	7.3 ± 1.27	P>0.1
Expt. GR- 3	8.75 ± 0.86	P>0.1
Control GR	7.3 ± 1.25	

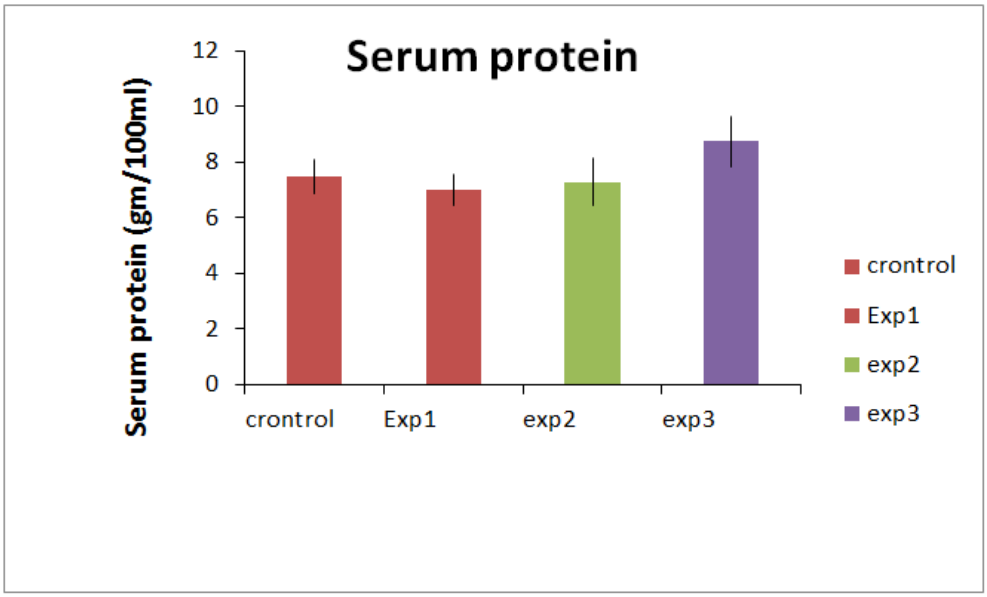

Figure 3: Graphical representation of relative changes of Total Serum Protein in different groups.

D) Changes in Blood Glucose

Table 4: Results showing the changes in Blood Glucose in different groups, treated with 1mg, 1.5mg and 2mg potassium chromate /100gm body weight/day, accordingly (GR 1-3) for consicutive 33 days. No significant change (P>0.1) was found when compared with control group. Values are expressed as means ± SD, N=5.

Group	Blood Glucose (mg/100ml) ± SD	P Values (Comparing with control)
Expt. GR- 1	91.29 ± 4.35	P>0.1
Expt. GR- 2	95.38 ± 3.62	P>0.1
Expt. GR- 3	96.62 ± 7.01	P>0.1
Control GR	90.18 ± 5.21	

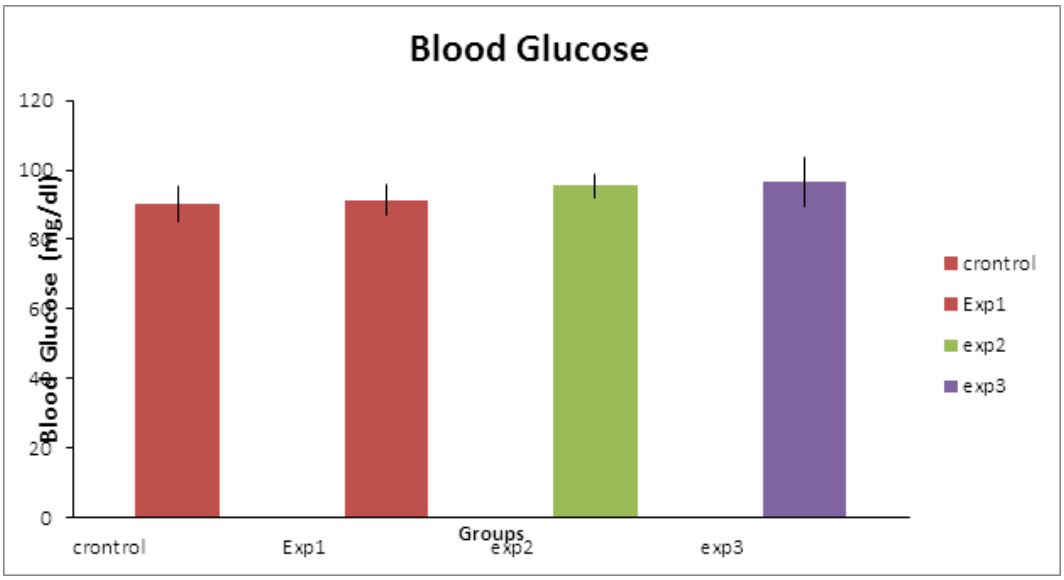

Figure 4: Graphical representation of relative changes of Blood Glucose level in different groups.

E) Changes in Alkaline Phosphatase Activity

Table 5: Results showing the changes in alkaline phosphatase activity in different groups, treated with 1mg, 1.5mg and 2mg potassium chromate /100gm body weight/day, accordingly (GR 1-3) for consicutive 33 days. No significant change (P>0.1) was found when compared with control group. Values are expressed as means ± SD, N=5.

Group	Alkaline Phosphatase activity (IU/l/ Min) ± SD	P Values (Comparing with control)
Expt. GR- 1	21.50 ± 2.12	P>0.1
Expt. GR- 2	22.25 ± 1.56	P>0.1
Expt. GR- 3	23.17 ± 2.77	P>0.1
Control GR	20.25 ± 2.86	

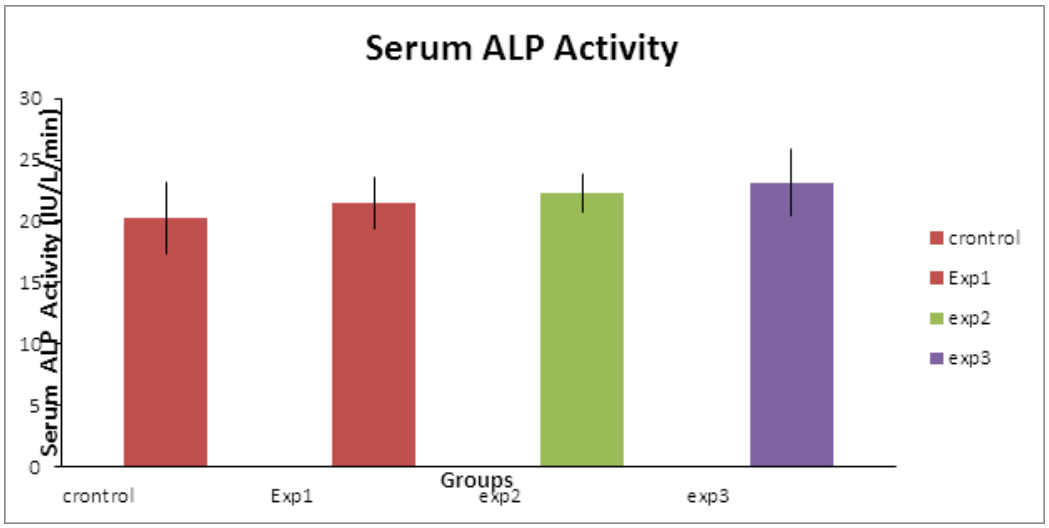

Figure 5: Graphical representation of relative changes of Alkaline Phosphatase activity in different groups.

F) Changes in Serum Glutamate Pyruvate Transaminase (SGPT) activity

Table 6: Results showing the changes in Serum Glutamate Pyruvate Transaminase activity in different groups, treated with 1mg, 1.5mg and 2mg potassium chromate /100gm body weight/day, accordingly (GR 1-3) for consicutive 33 days. No significant change (P>0.1) was found when compared with control group. Values are expressed as means ± SD, N=5.

Group	SGPT activity (IU/L/ Min) ± SD	P Values (Comparing with control)
Expt. GR- 1	41.95 ± 4.22	P>0.1
Expt. GR- 2	45.65 ± 4.73	P>0.1
Expt. GR- 3	44.9 ± 5.22	P>0.1
Control GR	46.10 ± 3.33	

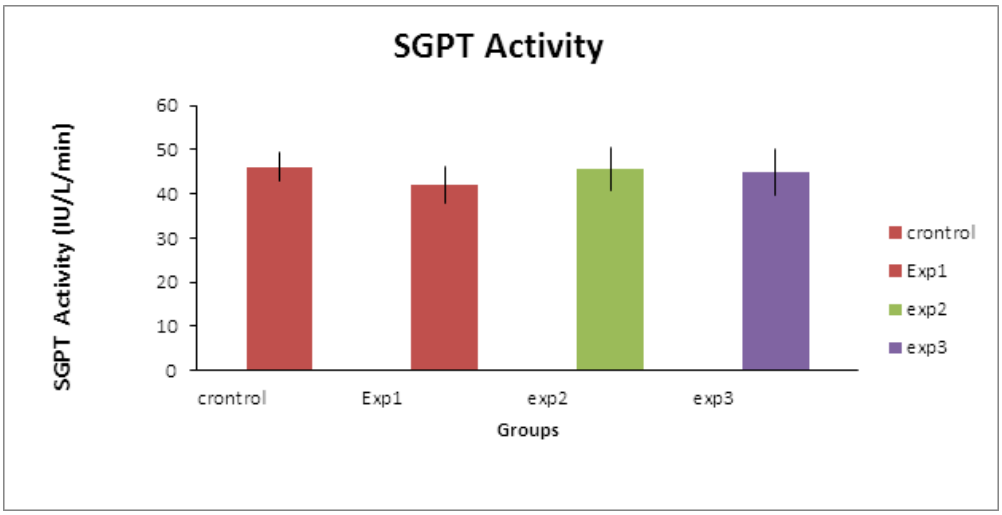

Figure 6: Graphical representation of relative changes of Serum Glutamate Pyruvate Transaminase (SGPT) activity in different groups.

G) Changes in Serum Glutamate Oxaloacetate Transaminase activity(SGOT) :

Table 7: Results showing the changes in Serum Glutamate Oxaloacetate Transaminase activity (SGOT) In different groups, treated with 1mg, 1.5mg and 2mg potassium chromate /100gm body weight/day, accordingly

(GR 1-3) for consicutive 33 days. No significant change (P>0.1) was found when compared with control group. Values are expressed as means ± SD, N=5.

Group	SGOT activity (IU/L/min) ± SD	P Values (Comparing with control)
Expt. GR- 1	71.65 ± 6.55	P>0.1
Expt. GR- 2	72.74 ± 7.21	P>0.1
Expt. GR- 3	79.45 ± 4.39	P<0.1
Control GR	65.98 ± 6.23	

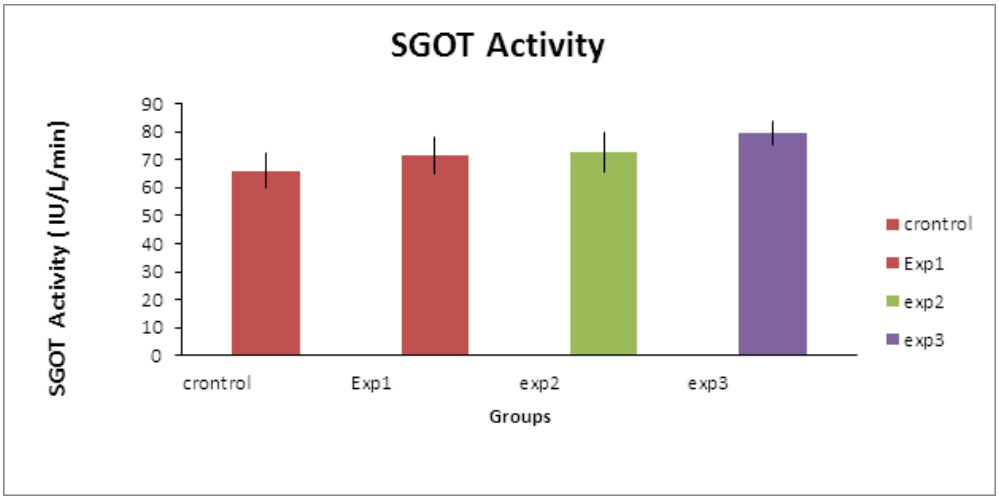

Figure 7: Graphical representation of relative changes of Serum Glutamate Oxaloacetate Transaminase activity (SGOT) in different groups.

DISCUSSION

Chromium (Cr) is an essential trace mineral that can improve our health benefits including insulin sensitivity, protein, carbohydrate and lipid metabolsm at very low doses i.e. 1mg per day for long term. Cr-III occurs naturally in soil and mineral deposits, while Cr-VI encountered in natural unpolluted soils. Cr-III is relatively immobile in soil or sediments. The most common form of Cr-VI is readily solubilised from most soil and transported through water that contacts the soils. Chromium and more particularly Cr-VI, have become prevalent environmental contaminants.[2] Although chromium (III) has been reported to be an essential nutrient, exposure to high levels via inhalation, ingestion or dermal contact may cause some adverse health effects. Accidental or intentional ingestion of extremely high doses of chromium(VI) compounds by humans has resulted in severe respiratory, cardiovascular, gastrointestinal, hematological, hepatic, renal, and neurological effects as part of the sequelae leading to death or in patients who survived because of medical treatment.[4,5]

In the present study, low doses of hexavalent chromium caused no marked haemochemical toxicity in male rats. As, no change in body growth rate by chromium suplim ent in rats indicated the notoxicity in common metabolic enzyme activities. But Bataineh et. al. in 1997, reported the reduced body weight in male mice by treatment with cr-vi at 42 mg / kg/day for 12 weeks.[13] Similar trends were also observed by Kanojia et. al. in 1998.[14] Such nontoxic effect was also reflected in blood hemoglobin level and serum total protein measurement. Fristedt et. al in 1965, reported the anemia in chrome plating workers swallowed planting fluid containing 300 g chromium trioxide.[15] Similarly Iserson et. al. in 1983 and Clochesy in 1984, reported the inhibition of blood coagulation by plasma protein depletion in a 17 years old male worker, who ingested high concentration of chromium salt accidentally.[16,17] The present serological observations are also may be referenced with the previos studies having minor haematological effects in animals after oral exposure to chromium VI, but no haematological effects were observed in animals after potassium dichromate in rats and mice, the only haematological effects consisted of slightly reduced mean corpuscular volume (MCV) and mean corpuscular haemoglobin(MCH) values.[18] The unalteration of blood glucose in chromium treated rats also further support the nontoxic effect of the selected doses of chromium concentration in the present study in male rats. This finding was also correlative with the findings of Andersan et al. 1997, provided the most definitive support for chromium supplementation in type II diabetes mellitus in a randomised, double blind, placebo-controlled study in china in 1997, where HbAlc levels were significantly lower at different doses of Cr(III) compared to placebo.[19] In 1987, Anderson, reported in humans and in animals, that chromium III, was anessential nutrient that plays a role in glucose, fat, and protein metabolism by potentiating the action of insulin and is also called glucose tolarance factor (GTF), which s a complex of chromium, nicotinic acid and possibly amino acids like glycine, cysteine and glutamic acid.[20] As the nutritional RDA of chromium is very safe for the long

term suplimentation, which was further studied by investigating the level of major marker enzymes like serum alkaline phosphatase, SGOT and SOPT in the present study. But the treatment of rats by gavage with 13.5 mg cr vi / kg Body wt./day as potassium chromate for 20 days showed changes and re-localisation of liver enzymes alkaline phosphatase, acid phosphatase, glucose 6 phosphatase.cholinesterase and lipase as determined by histochemical means.[18] Kaufman et al. in 1970, also reported marked elevation in SGOT and SOPT level when a 14 years old boy who died after accidental ingestion of chromium VI at an acute high dose.[21]

So, from the present study it may be summerised that the chromium at nutritional RDA concentratins are very much safe for prolonged use as nutritional supplement for treating in different nutritional deficiencies.

CONCLUSION

After oral supplementation of chromium (VI) in adult male albino rats for consicutive 33 days, it is found that chromium at nutritional RDA concentratins are very much safe for prolonged use as chromium supplementation at doses of 1mg, 1.5mg and 2mg /100gm body weight/day for cosicutive 33 days has no significant effect on body weight gain, Haemoglobin level, blood glucose level, serum protein, serum alkaline phosphatase activity and SGPT activity except a mild rise in SGOT.

REFERENCES

1. Kyle R A and Shampo M A. "Nicolas-Louis Vauquelin—discoverer of chromium". Mayo Clin. Proc, 1989, 64(6): 643.
2. "EPA updates CERCLA priority list of hazardous substances", The Hazardous Waste Consultant. 10(5), Mc. Coy and Associates, Inc., Lake wood, Colo, 2.26-2.30.
3. Lee KP, UirichCE, Geil RG et. al. Inhalation toxicity of chromium dioxide dust to rats aftr two yearsexposure. Sci Total Environ, 1989; 86: 83-108.
4. Mishra S and Bharagava R N, Toxic and genotoxic effects of hexavalent chromium in environment and its bioremediation strategies. Journal of Environmental Science and Health, Part C, 2016; 34(1): 1-32.
5. Tchounwou PB, Yedjou CG, Patlolla AK and Sutton DJ. Heavy metal toxicity and the environment, Experientia Supplementum, 2012; 101: 133-164.
6. Mathur J N. ICMR Bulletin, Natinal Centre for Laboratory Animal Sciences (NCLAS).A Profile, Published By The Indian Council of Medical Research, New Delhi, 2004; 34(4): 21-28.
7. Luma P and Vorne M. Changes in the hepatic microsomal enzyme activity during long-term ethanol feeding in rat. Acta Pharmacol. Toxicol, 1876; 138: 260-266.
8. Ida B. Andersen, Claus L. Brasen, Henry Christensen, Lene Noehr-Jensen, Dorthe E. Nielsen, Ivan Brandslund, Jonna S. Madsen. Standardised Resting Time Prior to Blood Sampling and Diurnal Variation Associated with Risk of Patient Misclassification: Results from Selected Biochemical Components. PLOS ONE, 2015; 10(10): 1-9.
9. Srivastava T, Negandhi H, Neogi SB, Sharma J, Saxena R. Methods for Hemoglobin Estimation: A Review of "What Works." Journal of Hematology & Transfusion, 2014.
10. Lowry OH. Rosebrough, NJ. Farr AL and Randall R.J. Protein measurement with the Folin phenol reagent. J. Biol., 1951; 193(1): 265-75.
11. Kind, PRN, and King, EJ . Estimation of Plasma Phosphatase by Determination of Hydrolysed Phenol with Amino-antipyrine. J Clin Path, 1954; 7(4): 322 - 326.
12. Reitman S and Frankel S, A Colorimetric Method for the determination of Serum Glutamic Oxalacetic and Glutamic Pyruvic Transaminases, Am J Clin Pathol, 1957; 28(1): 56-63.
13. Bataineh Hal-Hamood MH, Elbetieha A, Bani Hani I, "Effect of long term ingestion of chromium compounds on aggression, sexual behavior and fertility in adult male rat, " Drug Chem Toxicol, 1997; 20(3): 133-49.
14. Kanojia RK, Junaid M, Murthy RC, Embryo and Fetotoxicity of Hexavalent Chromium: A Long-Term Study, Toxicol Lett,, 1998; 95(3): 165-72.
15. Fristedt B, Lindqvist B, Schutz A, et al. Survival in a case of acute oral chromic acid poisoning with acute renal failure treated by haemodialysis. Acta Med Scand, 1965; 177: 153-159.
16. Iserson KV, Banner W, Froede RC, et. al. Failure of dialysis therapy in potassium dichromate poisoning. J Emerg Med, 1983; 1: 143 - 149.
17. Clochesy JM. Chromium ingestion : A case report. J Emerg Nursing, 1984; 10: 281 - 282.
18. Agency for toxic substances and disease registry (ATSDR), Atlanta, GA: U.S. Department of Health and Human Service, 2000.
19. Anderson RA, Cheng N, Bryden NA, Polansky MM, "Benificial effects of chromium for people with diabetes. J. Chi and J. Feng, 1997; 46: 1786-1791.
20. Anderson R. Chromium. In: Trace Elements in Human and Animal Nutrition (edited by Mertz M). Academic Press, San Diego, CA, 1987; 225-244.
21. Kaufman DB, DiNicola W, McIntosh R. Acute potassium dichromate poisoning: Treated by peritoneal dialysis. Am J Dis Child, 1970; 119: 374-376.