

## Hexavalent Chromium And Its Effects on Male Reproductive System of Albino Rats



### Zoology

**KEYWORDS :** Hexavalent chromium, albino rats, chromium-induced cytotoxicity.

**M. Santhosh Kumar**

Ph.D., Research Scholar, Post Graduate and Research Department of Zoology, Auxilium College (Autonomous), Gandhi Nagar, Vellore - 632006, Tamil Nadu

**C. Siva**

Ph.D., Research Scholar, Post Graduate and Research Department of Zoology, Auxilium College (Autonomous), Gandhi Nagar, Vellore - 632006, Tamil Nadu

### ABSTRACT

*Hexavalent chromium compounds are those that contain the metallic element chromium (Cr) in its hexavalent (positive-6) valence state. In this document, these compounds are denoted chromium (hexavalent compounds). In the present study, an attempt is made to know the chromium-induced cytotoxicity on the testis of pubertal albino rats. Since the reduction of chromium inside the cells, brings about various biochemical modifications, chromium-induced cytotoxicity on testis can be studied well by treating rats with chromium-mixed water. The general metabolism and steroidogenic processes of the testis, an attempt is made to know the impact of chromium on these testicular lipogenic enzymes. The direct effects of chromium-induced cytotoxicity on the testicular architecture, lipogenic processes of the testis and serum hormonal profiles in these pubertal rats in a dose-dependent manner. Due to these deleterious effects of chromium-induced cytotoxicity, the spermatogenic and steroidogenic processes in the testis may get impaired, leading to infertility.*

### Introduction

Chromium is a naturally occurring element found in rocks, volcanic dust and gases, soils as well as plants and animals. It is commonly used in three basic industries: metallurgical, chemical and refractory which leads to environmental pollution [6]. Chromium can exist in several oxidation states ranging from -2 to +6, of which the trivalent (III) and hexavalent (VI) forms are of biological importance [13]. Chromium VI induced acute and chronic toxicity, neurotoxicity, dermatotoxicity, genotoxicity, carcinogenicity, immunotoxicity and general environmental toxicity [8, 15]. Male reproductive system comprises a pair of testes, epididymides and accessory sex glands. Testes are encapsulated ovoid organs consisting of seminiferous tubules separated by interstitial tissue. The toxic effects of drugs and environmental chemicals on the human reproductive system have become a major health problem. Incidences of chemically induced germ cell damage and sterility appear to be on the increase. Several chemical contaminants including heavy metals present in the environment, when occupationally exposed to man, affects sterility, evidencing oligospermia, azoospermia and germinal aplasia [1, 7]. In the present study we have examined the effects of treatment of chromium VI on different reproductive parameters of adult male albino rats. The examined parameters included weight and histological analysis of testis was studied.

### Materials and methods

#### Animals and reagents

Three-month-old healthy male albino rats (110–130 g) purchased from the central animal facility, Dr. A.L.M. Post Graduate Institute of Basic Medical Sciences, University of Madras, Taramani, Chennai – 600 113 and were used in the present investigation. The rats were maintained in a well-ventilated laboratory condition, with  $12 \pm 1$  hours light:  $12 \pm 1$  hours dark, natural light schedule. They were supplied with standard rat pellet diet (Gold Mohur, Hindustan Lever Limited, Bangalore, India), supplemented with Bengal gram and carrots as their diet and clean drinking water ad libitum. Chromium VI as potassium dichromate (K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>) and other chemicals and reagents were obtained from Sigma-Aldrich (USA). Rats were randomly divided into three groups: Group-A rats (Control) – receiving 1 ml of vehicle (double distilled water) alone, daily for 7 days. Group-B rats (Exp-I) – 1 ml of Potassium dichromate solution (K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>, M.W: 294.18) containing a dichromate solution dosage of 10 mg/kg body weight and Group-C rats (Exp-II) 1 ml of potassium dichromate solution (K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>, M.W: 94.18) containing a dosage of 20 mg/kg body weight of rat dissolved in double distilled water was given orally, daily for 7 days. Daily changes in the body weights of rats were recorded. The doses were selected from results of

a preliminary experiment. Rats were weighed daily throughout the experiment. After forty five days of treatment, animals were sacrificed by decapitation; the whole reproductive system was removed with the epididymis intact were dissected and weighed. Animals were cared for in compliance with the practice for the Care and Use of Animals for Scientific Purposes. The experimental protocols were approved by the Faculty Ethics Committee (Tamil Nadu, India).

### Analytical Method

#### Radio iodination of hormones

Highly purified iodination grade rat FSH (NIDDK-r-FSH-I-7; AFP 9864B) and LH (NIDDK-r-LH-I-7; AFP-9404B) were obtained from the National Institute of Diabetes & Digestive & Kidney Diseases (NIDDK), National Hormone and Pituitary Program, Baltimore, Maryland, USA were radio iodinated with carrier-free <sup>125</sup>I and used for the RIA of serum hormones. Iodination of these hormones with <sup>125</sup>I was performed according to the method of [5], as modified by [4], using chloramine-T as an oxidizing agent.

### Histological analysis

Histological studies of low and high doses of chromium-treatments demonstrated the testicular structures of both control and chromium-treated pubertal rats. Control animals showed normal cellular arrangement in the seminiferous tubule. On the other hand, seminiferous tubular shrinkage was observed in both low and high doses of chromium-treated rat testes. Sloughing of germ cells from seminiferous epithelium was observed in the testis of high dose chromium-treated rats for 7 days. Degenerative changes in the testis with the disintegration of spermatocytes with above observations were also reported [2]. These degenerative changes due to chromium-treatment in the testis resulted in spermatogenic arrest with tubular necrosis and degenerating Leydig cells [2].

Occupational exposure to chromium in man will lead to severe reproductive injury among the exposed persons [12]. Moreover, the degree of testicular damage due to chromium exposure is directly proportional to the dose applied in rats [11]. Different doses of chromium-treatments to rats has decreased the tubular diameter, nuclear size of testicular cells and reduction in cell population of spermatogenic cells [11]. There are visible disruption in the germ cell population in the seminiferous tubule with degenerative changes observed in Leydig cells [2]. In the present study also, the observed decreased levels of serum testosterone levels in both low and high doses of chromium-treated rats could have severely impaired the testicular histoarchitecture which has been clearly observed from the plates.

### Statistical Methods

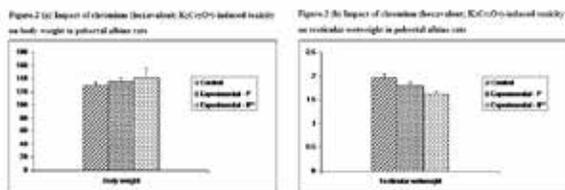
The data were analyzed statistically using Student's 't' test and expressed as Mean  $\pm$  standard Error of Mean (S.E.M) and was calculated as follows [10]. The value of probability (p) was obtained from the degree of freedom from the standard table given by [3]. If the calculated value was more than the table value, it was significant at that probability level. The following levels of significance were used.

$p < 0.001$  to  $p < 0.05$  considered as significant data and  $p > 0.05$  as non-significant data.

### Results

The impact of the different doses of chromium-treatment for 7 days and subsequent withdrawal effect for 5 weeks on the body weight and the total wet weights of testis in pubertal rats in depicted in (Figure-2 a & b). When compared to their age-matched control pubertal rats, chromium treatment (10 mg/kg body weight; Group B) for 7 days and subsequent withdrawal for 5 weeks has brought out a significant increase ( $p < 0.01$ ) in their body weight. This increase in bodyweight has increased to a further significant level ( $p < 0.001$ ) in Group C (20 mg/kg body-weight / 7 days) animals when compared to their age-matched controls (Figure-2 a & b). The wet weights of testis in both low dose (Group B) and high dose (Group C) chromium-treated rats failed to elicit any significant alteration in both the groups when compared to their age-matched control pubertal rats (Figure-2 a & b).

### Figure.2 Impact of chromium (hexavalent; K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>)-induced toxicity on body weight and testicular wet weights in pubertal albino rats

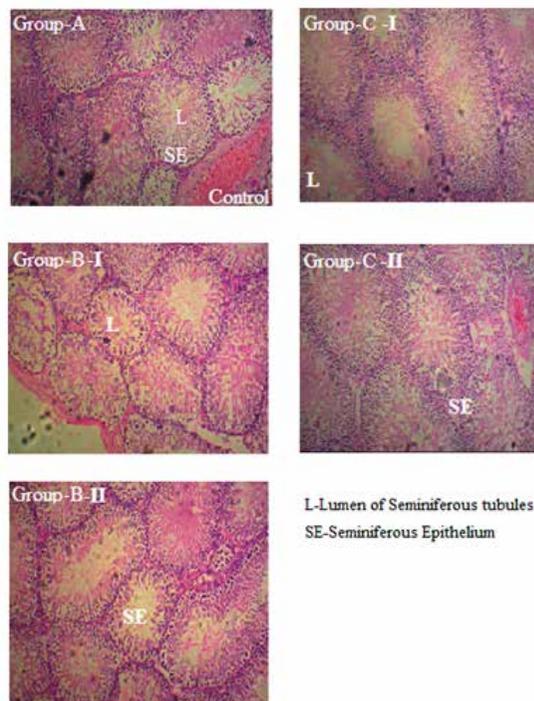


### Testicular Histology

The histology of testis in both low dose (10 mg/kg bodyweight) and high dose (20 mg/kg bodyweight) chromium-treated pubertal rats are depicted in Figure.1. Group A, B and C. The photomicrographs of testicular architecture in both control (Group-A), experimental-(Group-B & C) were compared. While in the control pubertal rats, the testicular histology show clear seminiferous tubules with the epithelium intact and there are varying stages of spermatids attached with the seminiferous epithelium. In the Experimental-B group of rats, chromium-treatment has indicated visible disruption in germ cell arrangements near the wall of seminiferous tubules and a decrease in the diameter of the seminiferous tubules.

In the Experimental-C group of rats, chromium-treatment has brought about the disruption of seminiferous epithelium along with the disruption in the attachment of sperm with the seminiferous epithelium. In these groups of rats, it was further observed the presence of intraepithelial vacuoles among the disrupted cells. Abnormalities affecting nearly all stages of germ cell development were seen in the seminiferous tubules, with narrowing up of the lumen of seminiferous tubules and necrotic mass of germ cells. The germ cells seem to be displaced towards the lumen of seminiferous tubules.

### Figure. 1 Chromium (Hexavalent; K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>) treatment – Testicular Histology of pubertal rats



### Discussion

Moreover, testosterone secreted from the Leydig cells of testis, is responsible for the activation and maintenance of spermatogenesis [14]. Testosterone helps in the maturation of round elongated spermatids by promoting the conversion of round spermatid between stages VII and VIII of the spermatogenic cycle [10]. In the present study also, the observed decreased levels of serum testosterone levels in both low and high doses of chromium-treated rats could have severely impaired the testicular histo-architecture which has been clearly observed from the plates.

### Conclusion

In conclusion the present data suggest the direct effects of chromium-induced cytotoxicity on the testicular architecture, lipogenic processes of the testis and serum hormonal profiles in these pubertal rats in a dose-dependent manner. Due to these deleterious effects of chromium-induced cytotoxicity, the spermatogenic and steroidogenic processes in the testis may get impaired, leading to infertility.

**REFERENCE**

1. Aruldhas, M.M., Subraminan, S., Sekar, P., Venkatesh, G., Chandrahasan, G., Govindarajulu, P and Akbarsha, M.A. (2005), Chronic Chromium exposure - induced changes in testicular histoarchitecture are associated with oxidative stress: study in a non-human Primate (*Macaca radiata*, Geoffroy) Human Reproduction, 20(10): 2801. | | 2. Chandra, A.K., Chatterjee, A., Ghosh, R. and Sarkar, M. (2007), Effect of Curcumin on Chromium-induced oxidative damage in male reproductive system, Environmental Toxicology and pharmacology, 24:160-166. | | 3. Fisher, R.A. and Yates, T (1948), In: Statistical tables for biological, agricultural and medical research, Oliver and Boyd, London. | | 4. Greenwood, F.C., Hunter, W.M. and Clover, J.S. (1963), The Preparation of <sup>131</sup>I-labeled human growth hormone of high specific radioactivity, Biochem.J. 89: 114-123. | | 5. Hunter, W.M., and Greenwood, F.C. (1962), Preparation of <sup>131</sup>I-labeled human growth hormone of high specific activity. Nature, 194:495. | | 6. Kumar, R., (1987) Environmental pollution and health hazards in India. Ashish Publishing house, New Delhi, India. | | 7. Kumar, S., Sathwara, N.G., Gautam, A.K. Agarwal, K. Shan, B., Kulkarni, P.K. Patel, A., Dave, L.M., Parikh, D.J. and Saiyed, H.N. (2005). Semen quality of industrial workers occupationally exposed to chromium. J.Occup.Health, 47:424. | | 8. Li, Z.H., Li, P., Randak, T., (2011) Evaluating the toxicity of environmental concentrations of waterborne chromium (VI) to a model teleost, *oncorhynchus mykiss*: a comparative study of in vivo and in vitro. Comparative Biochemistry and Physiology Part C 153 402-407. | | 9. O'Donnell, L., McLachlan, R.L., Wreford, N.G., Robertson, D.M. (1994), Testosterone promotes the conversion of round spermatids between stages VII and VIII of the rat spermatogenic cycle, Endocrinology, 135 (6), 2608-2614. | | 10. Ostle, B. (1966). In : Statistics in Research, Oxford and IBH Publications, New Delhi. | | 11. Roy Chowdhury, A. and Mitra.C. (1995), Spermatogenic and steroidogenic impairment after chromium treatment in rats, Ind. J. Exp. Biol 33, 480-4. | | 12. Roy Chowdhury, A. (2009), Recent Advances in Heavy Metals Induced Effect on Male Reproductive function - A Retrospective, Al. Ameen, J.Med. Sci. 37-42. | | 13. Stoecker, J.B., (2004) Chromium. In Elements and their compounds in the environment. Eds Merian E, Anke M, Ihnat M, Stoepler M. Copyright, Weinheim, Germany. 2nd edition, pp 709-729. | | 14. Singh., O' Neill, C., Handelsman, D.J. (1995), Induction of Spermatogenesis by androgens in gonadotropin - deficient (HPG) mice, Endocrinology; 136: 5311-5321. | | 15. Von Burg, R., Liu, D., (1993) Chromium and hexavalent chromium. Journal of Applied Toxicology 13 225-230.