Potassium Dichromate Impact on Male Reproductive Integrity Biomarker in Rat. Two Generation Study

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Abstract
The aim of this study was the evaluation of Cr VI cumulative and differentiate exposure on integrity biomarker of male reproductive system. The objective was evaluation of potassium dichromate intake on genital organs and sexual accessory glands architecture in male rats from two generation. Males and females from F_0 generation were exposed to potassium dichromate three months before mating. F_1 generation was represented by male pups, resulted from F_0 generation, exposed to potassium dichromate *in utero*, during suckling (via milk and water) period and until sexual maturity. All the animals were divided in one control and three experimental groups, exposed to Cr VI trough drinking water, as followed E_1: 25 ppm (LOAEL); E_2: 50 ppm: E_3: 75 ppm; control group received tap water without chromium content. The experiment was carried out with respecting legislation regarding ethics in animal experiments. The study revealed the presence of congestive and degenerative lesions in genital organs and sexual accessory glands of exposed individuals from F_0 and F_1 generation such as: interstitial edema, epithelial necrosis and atrophy, membrane exfoliation and necrosis. The lesions frequency and intensity were directly correlated to exposure level, the most affected being E_3 group and generation, more pronounced in F_1 generation.

Keywords: chromium VI, histoarchitecture, male, rat, toxicity

1. Introduction

Worldwide distribution and extensive use of chemical agents is associated with concern of the highest priority for environmental and occupational exposure which may have dramatic effects on male reproductive function. Chromium has been identified to be one of these toxic metals [1]. Chromium is found in the environment in soil, rocks, animals and plants [2]. In general, in environment, chromium exists in three main oxidation forms chromium (0), chromium (III) and chromium (VI) [3].
A number of researches have indicated that chromium (III) plays an important role in normal protein, fat and carbohydrate metabolism, as well as improves insulin sensitivity [4].

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Chromium VI is more toxic than in trivalent form because it readily enters the cells producing various pathological conditions, including reproductive dysfunction [5].
Chromium (III) and chromium (VI) compounds are widely used industrially in stainless steel production, welding, electroplating, leather tanning, production of dyes and pigments and wood preservatives [2].
Chromium (VI) occurs mostly due to anthropogenic origin and is considered a human carcinogen [2] and is known to cause in humans and experimental animals hepatotoxicity, nephrotoxicity, too [6].
In the cell, Cr (VI) is converted to more stable Cr (III) with the production of reactive oxygen species (ROS), responsible for production of oxidative stress. Most of the chromates that induce toxicity provoke lipid peroxidation, DNA damage, cytotoxicity, mutagenesis and carcinogenesis [7].

Different studies demonstrated Cr (VI) as a major risk factor to growing testis [8] and also for adult testis [9]. Testicular tissues are major target organ for metals that induce oxidative damage because of its high contents of polyunsaturated membrane lipids [10].

The aim of this study was the evaluation of Cr VI cumulative and differentiate exposure on integrity biomarker of male reproductive system. The objective was evaluation of potassium dichromate intake consequences on genital organs and sexual accessory glands architecture in male rats from two generation.

2. Materials and methods

The study was carried out on two generation male rats. F_0 generation was represented by 28 White Wistar male rats divided in three experimental and control group. Rats were exposed to potassium dichromate for three months before mating as followed: E_1: 25 ppm (LOAEL) [11]; E_2: 50 ppm (2 x LOAEL); E_3: 75 ppm (3 x LOAEL); control group received tap water without chromium content.

After mating with female rats exposed to potassium dichromate for the same period of time and Cr VI level all males were sacrificed following protocols and ethical procedures. Genital organs and sexual accessory glands were collected and histological examined after staining with Hematoxylin and Eosin (H.E.) method.

Female rats continued to be exposed during gestation and lactation period to same levels of Cr VI. After weaning, male pups (F_1 generation) were separated from female pups. Male pups continued to be exposed to same levels of Cr VI trough drinking water for three more months (until sexual maturity). After this period of time seven rats from every group were sacrificed following protocols and ethical procedures. Genital organs and sexual accessory glands were collected and histological examined after staining with Hematoxylin and Eosin method.

All the animals were provided free access to food and water.

The study was performed in compliance with national and international law regarding animal welfare and ethics in animal experiments: 143/400/2002; 471/2002; 205/2004; 206/2004; 9/2008; 86/609/CEE.

The results were statistically analyzed by Anova method and Student test.

3. Results and discussion

Consecutive chromium exposure and accumulation in genital organs and sexual accessory glands severe structural modifications appeared.

The structural changes appeared in F_0 generation are presented in figures 1 - 4, and those form F_1 generation in figures 5 - 10.

The histological changes were:

- *in testes:* interstitial edema, seminiferous tubules necrosis, seminiferous tubules membrane necrosis and exfoliation, seminiferous tubules epithelial atrophy, Leydig cell necrosis, Sertoli cell necrosis;
- *in epididymis:* interstitial edema, epithelial necrosis, basal membrane exfoliation, epithelial smoothing;
- *in prostate, seminal vesicles and bulbo-urethral glands*: epithelial necrosis, membrane exfoliation.

Other authors who observed similar structural changes after animal exposure to Cr VI were: Chandra *et al.* [12], Chowdhury *et al.* [13], Li *et al.* [14], Murthy *et al.* [15] and Muselin *et al* [16].

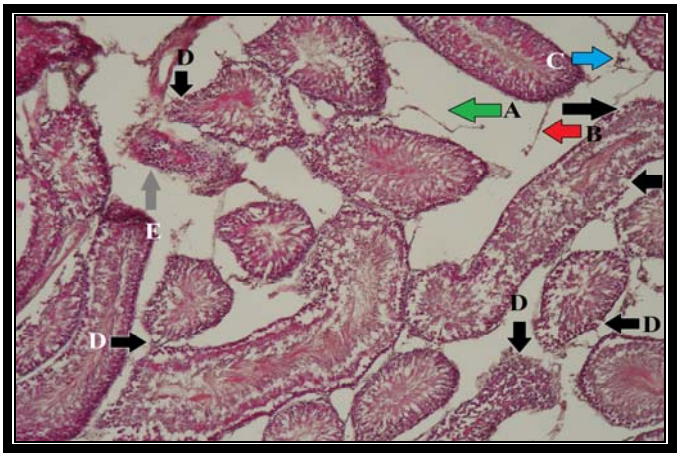

Figure 1 Testis section (75 ppm, H.E. stain 100x) A- interstitial edema, B – seminiferous membrane exfoliation, C – Leydig cell necrosis, D – seminiferous epithelium atrophy and necrosis, E – seminiferous tubules necrosis

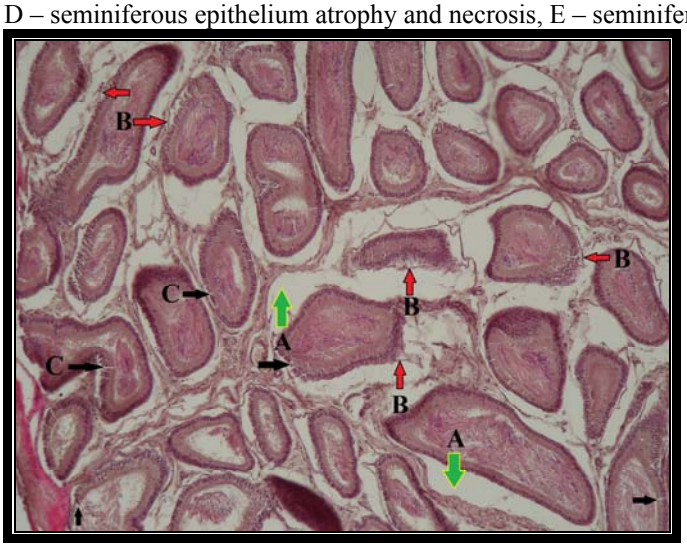

Figure 2 Epididymis section (50 ppm, H.E. stain, 100x) A – interstitial edema, B – basal membrane exfoliation, C – epithelial necrosis

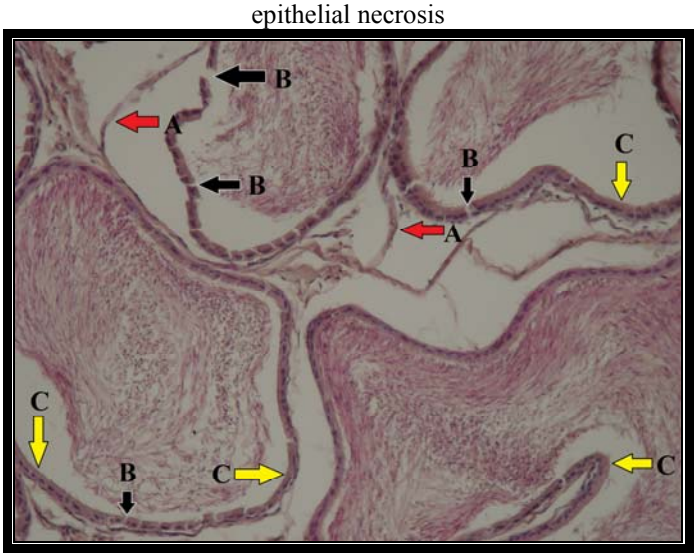

Figure 3 Epididymis section (75 ppm, H.E. stain, 400x) A – membrane exfoliation, B – epithelial and basal membrane necrosis, C – epithelial smoothing

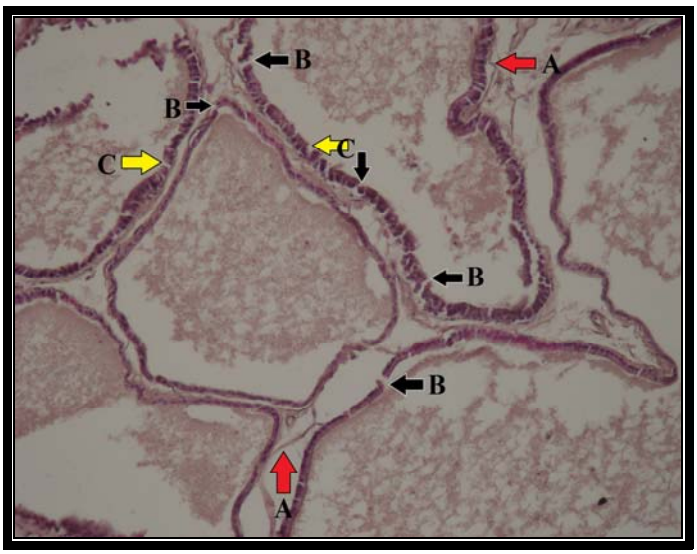

Figure 4 Prostate section (75 ppm, H.E. stain, 400x) A – membrane exfoliation, B – epithelial partial necrosis, C – epithelial complete necrosis

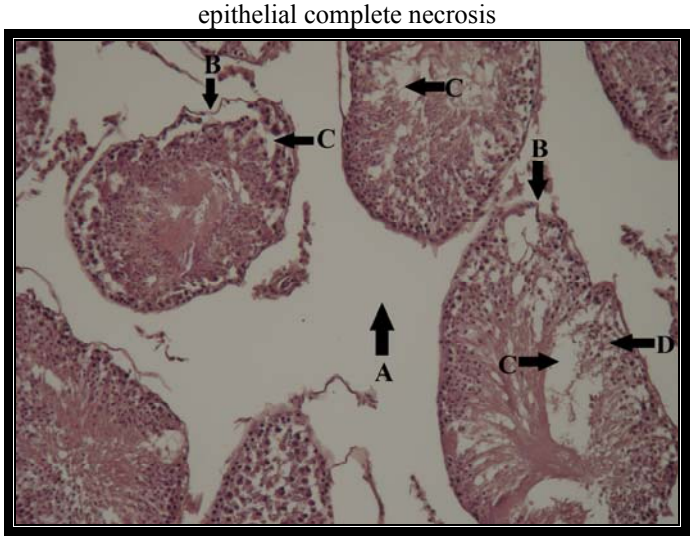

Figure 5 Testis section (75 ppm, H.E. stain, 200x) A – interstitial edema, B - seminiferous membrane necrosis and exfoliation, C – total destruction of germinal cells, D – necrosis of germinal cells

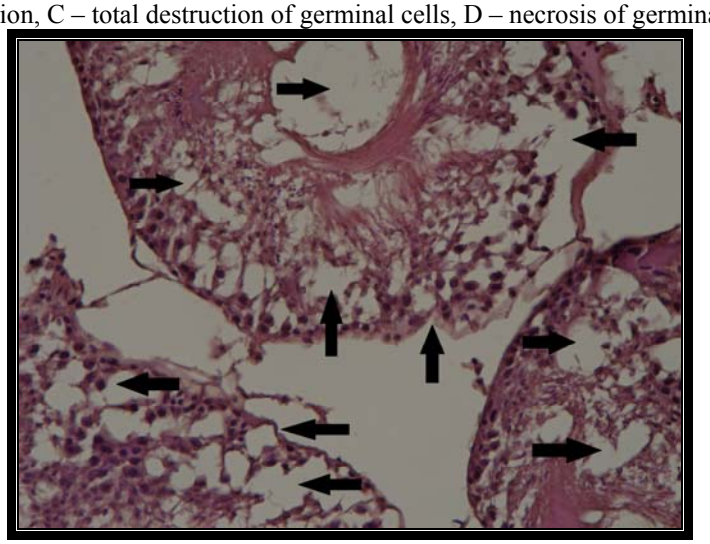

Figure 6 Testis section (75 ppm, H.E. stain, 400x) arrows show different all the structures in stages of degeneration (in general total necrosis)

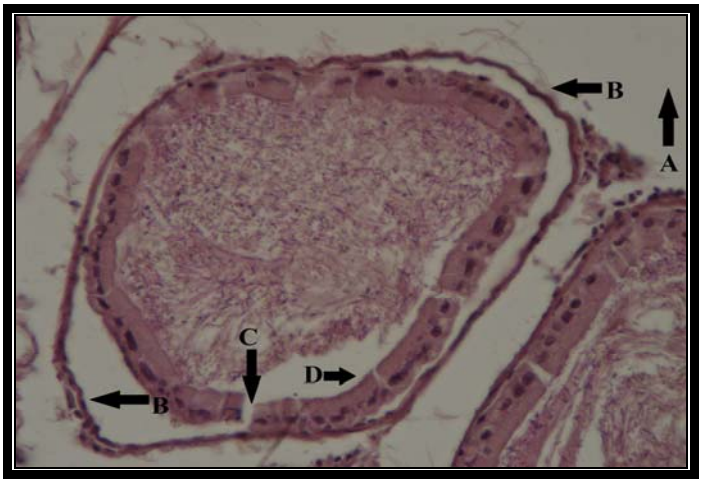

Figure 7 Epididymis section (25 ppm, H.E. stain, 400x) A – interstitial edema, B –total membrane exfoliation, C – epithelial necrosis, D – epithelial smoothing

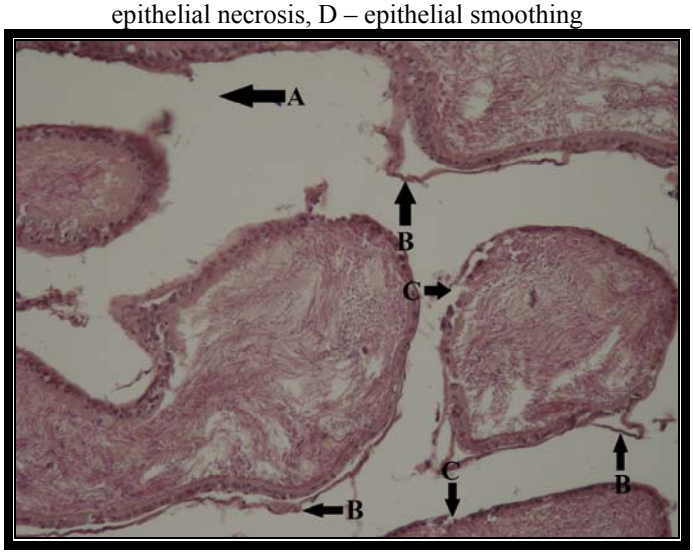

Figure 8 Epididymis section (50 ppm, H.E. stain, 400x) A – interstitial edema, B – membrane exfoliation, C – epithelial and membrane total necrosis with loss of the basal cells

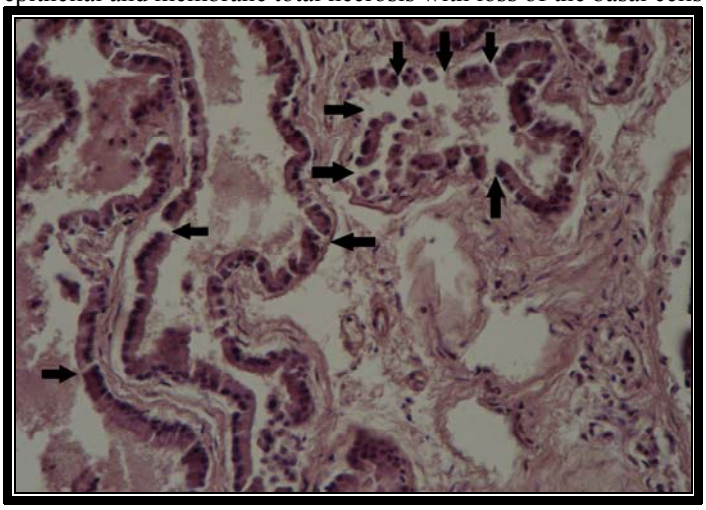

Figure 9 Prostate section (75 ppm, H.E. stain, 400x) arrows indicate necrosis of epithelium; epithelial cells are fallen in lumen

4. Conclusions

Exposure to different levels of potassium dichromate during two generation induced: congestive and degenerative lesions in male rat's genital organs and sexual accessory glands, directly correlated to exposure level and duration (more pronounced in F_1 generation).

Acknowledgements

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