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Original Research

Biochemical and Pathomorphological Study of Potassium Dichromate-induced Nephrotoxicity in Wistar Rat

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Abstract

An experiment was conducted to study biochemical and pathomorphological alterations induced by potassium dichromate toxicity. Forty colony bred Albino Wistar strain rats of both sexes, divided uniformly into four equal groups Group A, Group B, Group C, and Group D. Each Group contains five male and five female. Group A rats received only deionised water and served as control. Group B (low dose), Group C (mid dose) and Group D (high dose) rats were given potassium dichromate orally by gavage for 28 days at the rate of 0.625 mg/kg body weight (b.wt.), 1.25 mg/kg b.wt. and 2.5 mg/kg b.wt. respectively. A dose dependant significant rise in plasma alanine aminotransferase (ALT), plasma aspartate aminotransferase (AST), plasma alkaline phosphatase (ALP), creatinine and blood urea nitrogen was observed in treatment group, whereas, a significant decrease in total protein and albumin was observed in treatment group. Histopathological sections of kidney, liver, lung and testes revealed varying degrees of congestion, haemorrhage, degeneration and necrosis in rats of different treatment groups. The present study indicates nephric and hepatic toxicity in albino wistar rats due to potassium dichromate toxicity.

Keywords: Biochemical; Histopathology; Potassium dichromate; Rat

Introduction

The problem of environmental pollution is one of the most burning topics and has been on the checklist of almost all the nations. Among these environmental pollutants, heavy metals such as lead, mercury, arsenic, chromium and cadmium have received special attention worldwide as they are widely distributed in nature and leads to widespread occurrence of specific toxicological problems (Patra and Swarup, 2000). Chromium is a naturally occurring element found in volcanic dust, rocks, soil, plants, and animals. The most common forms of chromium in the environment are hexavalent (Cr^{6+}) or chromate, and trivalent (Cr^{3+}) or chromites. Cr^{6+} are widely used in industrial and chemical processes, such as leather tanning, printing, in hair dyes, steel manufacturing, and wood preservative production. In some regions, waste disposal of chromium compounds to the environment contributes to increase its presence and potential toxicity. Chromium induces dermatotoxicity, immunotoxicity, neurotoxicity, genotoxicity, and carcinogenicity (Bagchi *et al*., 2002). Chromium compounds induce oxidative stress leading to tissue damage (Stohs *et al*., 2001). Kawanishi *et al*., (1986) demonstrated that reactive oxygen species (ROS) including superoxide anion, singlet oxygen and hydroxyl radicals are generated during reduction process might be responsible for Cr (VI) toxicity. The present work was carried out to study biochemical and histopathological alterations induced by potassium dichromate toxicity.

Materials and methods

Experimental animals

The study was conducted on fifty colony bred Albino Wistar strain rats of both sexes, which were procured from Cadilla Pharmaceuticals, Dholka,

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Ahmadabad , Gujarat, India and were maintained under standard management conditions. Males and females were separated and all the animals were quarantined and acclimatized to the laboratory conditions of 12 hours day and 12 hours night. The animals were provided with standard pelleted food and water ad lib. The Institutional Animals Ethics Committee (IAEC) approved the experimental protocol vide letter No. SDAU/DVC/VSR/IAEC /7992-8001/08 dated 6th June 2008. The experimental protocol met the national guidelines as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Experimental design

Rats were divided uniformly into four equal groups Group A, Group B, Group C and Group D. Each group contains five male and five female. Group A rats received only deionised water and served as control. Potassium dichromate was administered orally by gavage for 28 days to Group B (low dose), Group C (mid dose) and Group D (high dose) rats were given at the rate of 0.625 mg/kg b.wt., 1.25 mg/kg b.wt. and 2.5 mg/kg b.wt. respectively. LD_{50} of potassium dichromate is 25 mg/kg b.wt.

Collection of Blood samples

Rats were anesthetized by using diethyl ether and blood was collected from all experimental groups on 28th day of experiment from retro-orbital plexus with the help of capillary tube in a heparinized vial (10 I.U.) for biochemical estimations. Plasma was separated from heparinised blood for biochemical estimations.

Collection of tissues specimens

Rats were sacrificed on 29th day of post-treatment and tissues from various organs were collected in 10 percent neutral buffered formalin. Tissue samples viz., liver, lung, kidney, stomach, intestine, spleen, thymus, skin, heart, brain and testis were collected for histopathological study.

Biochemical assay

All the biochemical parameters were analysed using Mercks Kits (Mercks Specialities Private Ltd., Mumbai-400018, India) by Clinical Analyzer (Systronics, Ahmedabad).

Pathomorphology

After recording the gross lesions, the tissues from affected lung, liver, brain, heart, spleen, kidney, stomach, intestine and testis were collected from sacrificed animals and subsequently preserved in 10 percent neutral buffered formalin for at least 24-48 hours. Further these tissues were processed by routine method of dehydration in graded alcohol, clearing in xylene and embedding in paraffin. Sections of 5-6μ thicknesses were prepared and processed by routine Hematoxylene and Eosin method to study the general histopathological alterations (Luna, 1968).

Statistical analysis

The statistical analysis of data generated on various parameters was subjected to statistical analysis using completely randomized design (CRD) (Snedecor and Cochran, 1980) and using CD values compared the treatment means. Since, the CD permits comparison of two consecutive treatment mean after arranging treatment mean in ascending or descending order, it was thought worthwhile to compare treatment mean with all other treatment mean (Overall comparison). Hence, Duncan's New Multiple Range Test (DNMRT) (Steel and Torrie, 1984) was used for the same.

Results

Biochemical parameters in serum studied for all the animals were shown in Table 1. A dose dependant significant ($P < 0.05$) rise in mean values of plasma alanine aminotransferase, plasma aspartate aminotransferase, plasma alkaline phosphatase, creatinine and blood urea nitrogen was observed in Group C and Group D on 28th day post treatment as compared to control Group A rats. A significant ($P < 0.05$) decrease in mean values of total protein and albumin was observed in Group C and Group D. No significant difference was observed in globulin value of all the treatment groups when compared with control Group A rats.

All the rats exposed to potassium dichromate at three different dose levels revealed dose dependant

Table 1. Serum biochemical parameters (mean±SE) of Wistar rats exposed to potassium dichromate.

Parameters	Groups			
	Group A (Control)	Group B (Low dose)	Group C (Mid dose)	Group D (High dose)
ALT (IU/L)	44.664±3.065^{a}	48.920±2.211^{ab}	62.512±1.475^{c}	69.067±1.082^{cd}
AST (IU/L)	173.33±4.980^{a}	186.38±4.574^{ab}	198.62±4.490^{bc}	210.11±4.538^{cd}
ALP (IU/L)	77.632±2.387^{a}	85.791±2.802^{ab}	90.307±1.811^{bc}	101.945±3.044^{d}
Creatinine(mg/dl)	0.527±0.020^{a}	0.625±0.020^{a}	0.814±0.025^{b}	1.122±0.030^{c}
BUN (mg/dl)	17.73±0.285^{a}	18.4±0.155^{b}	22.29±0.204^{c}	27.44±0.240^{d}
Total Protein (g/dl)	6.25±0.321^{a}	5.74±0.220^{a}	4.93±0.279^{b}	4.19±0.204^{c}
Albumin (g/dl)	3.62±0.244^{a}	3.44±0.137^{a}	2.45±0.209^{b}	1.90±0.111^{bc}
Globulin (g/dl)	2.62±0.138^{a}	2.22±0.109^{a}	2.48±0.138^{a}	2.28±0.143^{a}

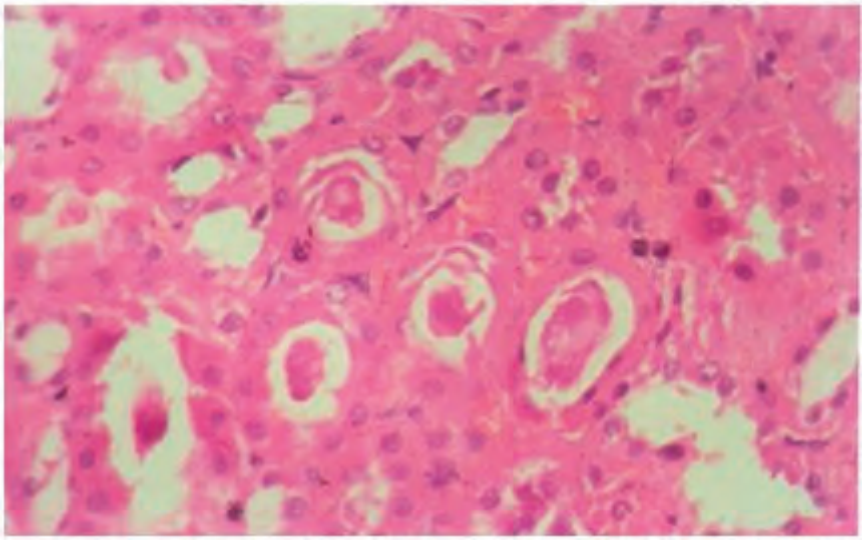

Fig. 1. Section of kidney showing severe tubular degeneration along with presence of renal cast in lumen of tubules in Group D.

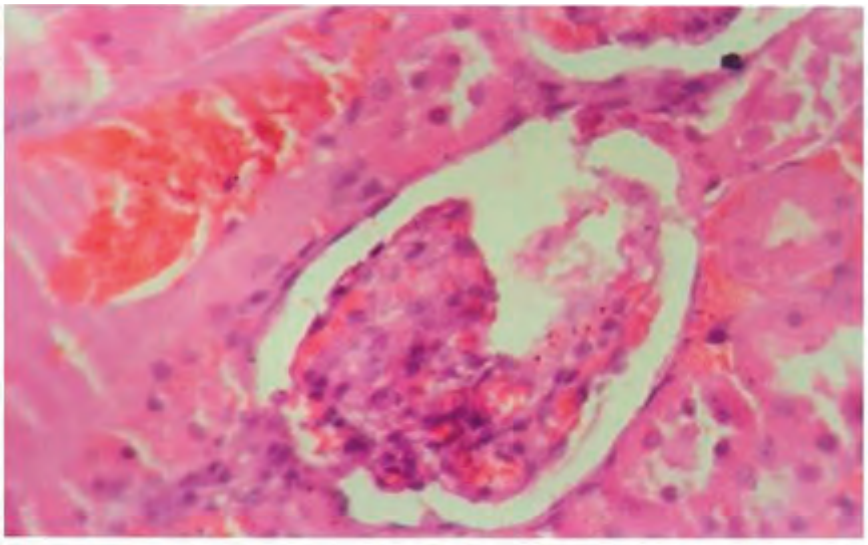

Fig. 2. Section of kidney showing severe glomerular hemorrhages along with increased Bowman's space in Group D.

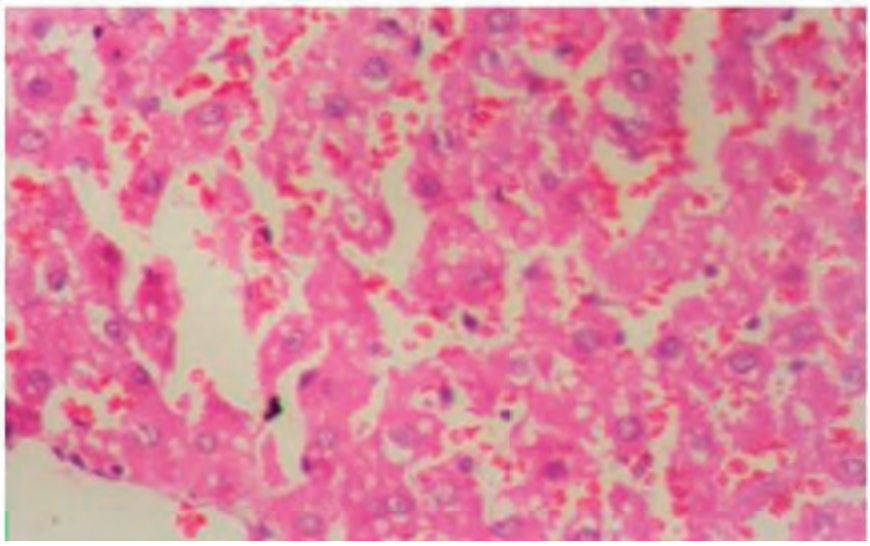

Fig. 3. Section of liver showing vacuolation along with sinusoidal dilatation and sinusoidal haemorrhages in Group C.

pathological changes. The pathomorphological changes comprised of varying degrees of congestion, hemorrhages, degeneration and necrosis in various visceral organs. The lesions were severe and predominant in kidney followed by liver. Renal lesions were consisted mainly of massive tubular hemorrhages, congestion, severe tubular degeneration along with presence of renal cast in lumen of tubules (Fig.1), severe glomerular hemorrhages along with increased Bowman's space (Fig.2), whereas, liver showed fatty changes along with sinusoidal dilatation and sinusoidal haemorrhages (Fig.3). The severity and distribution of such lesions were found higher in rats of Group C and Group D. lungs of Group C and D rats revealed emphysema characterized by distention and dilatation of alveoli along with thickening of interalveolar septa with presence of mononuclear cells and erythrocytes (Fig.4). Testes of Group C and Group D rats showed moderate to severe testicular degeneration with emptying of the seminiferous tubules along with increased inter tubular space. Intestine of Group D rats revealed desquamation and denudation of surface epithelium of the villi leading to erosions, ulcerations and necrosis.

Discussion

The mean values of plasma alanine aminotransferase, plasma aspartate aminotransferase, plasma alkaline phosphatase, creatinine and blood urea nitrogen revealed dose dependant significant ($p < 0.05$) increase in Group C and Group D rats when compared with mean value of Group A control rats. It suggests that oral administration of potassium dichromate at various dose levels has significant effect on kidney and liver functions. However, there was dose dependent significant rise in the serum levels of Creatinine, Urea, ALT, AST and ALP indicating pathological changes in the renal and hepatic system of significant nature. There was dose dependent reduction in the total protein and albumin. This could be related to intestine and liver changes. Similar results were also observed in rats (Pedraza *et al*., 2005; Fatima and Mahmood, 2007; Shelar, 2007; Srinivasan *et al*., 2008; Vihol *et al*., 2012).

Hexavalent chromium is readily taken up into tissue and is reduced inside the cell to the trivalent chromium. This reduction process causes the generation of reactive oxygen species which are involved in renal damage (Pedraza *et al*., 2005). The nephorotoxic lesions of potassium dichromate as observed in the present study were also reported by various workers (Acharya *et al*., 2001; Perez *et al*., 2004; Pedraza *et al*., 2005; Oliveira *et al*., 2006; Shelar, 2007; Zhou *et al*., 2008; Vihol, 2012). Liver showed fatty changes along with sinusoidal dilatation and sinusoidal haemorrhages whereas, lungs of Group C and D rats revealed emphysema characterized by distention and dilatation of alveoli along with thickening of interalveolar septa with presence of mononuclear cells and erythrocytes. Elevated ROS level in chromium-treated mice induces impaired Leydig cell function, changes several biochemical indices of cells including lipid

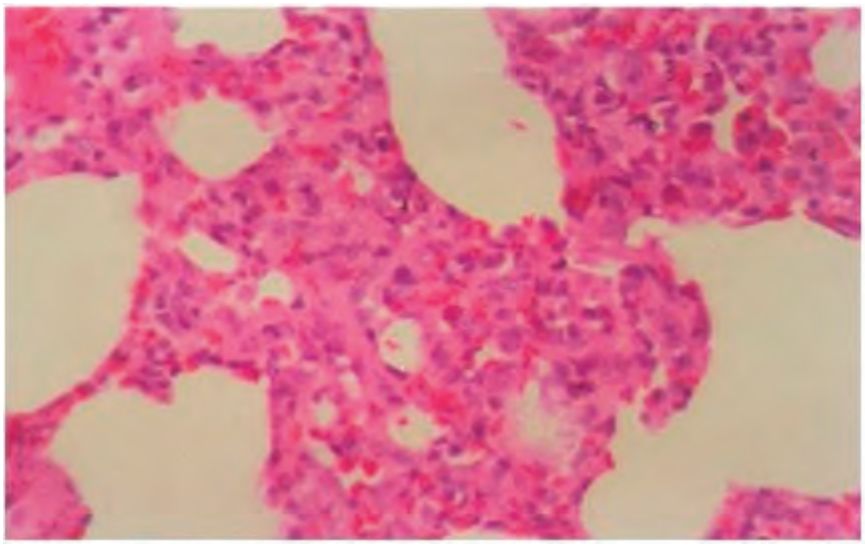

Fig. 4. Section of lung showing dilatation of alveoli along with thickening of interalveolar septa with presence of mononuclear cells and erythrocytes in Group D.

peroxidation (Chandra *et al*., 2007). Similar results were also observed in rats (Pedraza *et al*., 2005; Oliveira *et al*., 2006; Silva *et al*., 2006; Chandra *et al*., 2007; Shelar, 2007; Zhou *et al*., 2008; Rankov *et al*., 2010; Vihol *et al*., 2012).

Biochemical alterations and Pathomorphological lesions induced by potassium dichromate toxicity suggests that oral administration of potassium dichromate produce renal and hepatic toxicity in albino wistar rats.

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