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Original Article

Anatomy Section

Histopathological Changes in the Kidney of Albino Rat due to Chromium and the Ameliorative Role of α-Tocopherol

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ABSTRACT

Introduction: Potassium dichromate, a chromium {Cr(VI)} compound is the most toxic form of Cr(VI) which causes nephrotoxicity associated with oxidative stress in animals and humans. The study of toxicity and biological effects associated with chromium has generated a lot of interest due to its wider distribution in the environment and its use.

Aim: To study the potassium dichromate-induced nephrotoxicity and ameliorative role of vitamin E in albino rats.

Materials and Methods: The present experimental study was conducted in the Department of Anatomy, Subharti Medical College, Meerut, Uttar Pradesh, India. Study population consisted of 36 albino rats which were randomly divided into four equal groups (n=9, each). Group I served as control while groups II and III were administered Potassium dichromate ($K_2Cr_2O_7$) dissolved in sterile distilled water 10 mg/kg body weight single dose orally for 1, 14 and 42 days, 3 rats in each sub-groups. In addition to Cr(VI), group III also received α-tocopherol 125 mg/kg body weight daily orally. Group IV rats were maintained as α-tocopherol control and they received α-tocopherol as above daily for 42 days. At the end of 1, 14 and 42 days, all the rats were sacrificed for the estimation of the histopathological changes in kidney. The Z-proportion test was used for evaluation of data.

Results: Thirt-six albino rats of both sex, age about 60 days weighing approximately 140±10 gm were studied. Prominent pathological changes like glomerular mesangial proliferation and obliteration of bowman's space were observed in the kidney of group II rats. Co-treatment with α-tocopherol in group III significantly reversed the Cr(VI) induced changes with a p-value=0.009 and 0.007 for glomerular mesangial proliferation and obliteration of bowman's space respectively.

Conclusion: The α-tocopherol exhibited protective effect against Cr(VI) induced damage to the kidney.

Keywords: Glomerular mesangial proliferation, Obliterated bowman's space, Potassium dichromate, Vitamin E

INTRODUCTION

Living organisms require varying amount of heavy metals like iron, zinc etc., for their survival [1]. Excessive levels of these metals can be damaging to the organism. Other heavy metals like chromium, lead etc., are toxic metals and their accumulation over time in the bodies of animals can cause serious illness [2].

Chromium is a naturally occurring element found in volcanic dust, in earth crust and is widely distributed in air, water, rocks, soil, plants and animals [3]. Chromium exists in a series of oxidation states from -2 to +6 valence. The Cr(III) and Cr(VI) forms are of biological significance. The Cr(III) occurs naturally in the environment and is an essential nutrient, while Cr(VI) and Cr(0) are generally produced by industrial and chemical processes such as in leather tanning, printing, stainless steel manufacturing and in wood preservative production [4].

The Cr(VI) is the major terrestrial pollutant and is highly toxic. They induced dermatotoxicity, immunotoxicity, neurotoxicity, genotoxicity and carcinogenicity [5]. Nephrotoxicity with increased urinary β-2 microglobulin and acute tubular necrosis has been reported in ferrochromate workers exposed acutely and chronically to Cr(VI) [6].

As chromium excretion is mainly through the renal route, so acute exposure of potassium dichromate in rats increases the chromium content in kidney [7]. Chromium has been found to be generating various free radicals like superoxide, nitrogen species (peroxynitrite, nitric oxide) and hydroxyl indirectly causing oxidative damage [8]. The Cr(VI) exposure has been shown to produce lesions at the level of the proximal tubular cell and Lipid Peroxidation (LPO) in human kidney [9,10]. In humans, accidental acute ingestion of Cr(VI) may lead to acute renal failure [11].

Reduction of Cr(VI) to Cr (III) results in formation of Reactive Oxygen Species (ROS) that induce oxidative damage [12]. This in turn is responsible for defective haematopoiesis and a cascade of cellular events including modulation of apoptosis regulatory gene P53 and contribute to the cytotoxicity, genotoxicity and carcinogenicity [13,14]. Although, the role of these species, has not been proved in renal toxicity. The injury associated with Cr(VI) has been seen to be mitigated by antioxidant supplementation suggests the role of oxidative stress related injury [15].

The most common and active form of vitamin E in-vivo is α-tocopherol [16,17]. It is an antioxidant which serves as an important lipophilic radical-scavenger. It is highly reactive to peroxyl radicals (10,000 times faster) compared to polyunsaturated lipids [18]. Hence, vitamin E is widely used as a therapeutic agent for the treatment of oxidative damage related disorders [19]. The LPO which are activated by heavy metals like dichromate gets reduced by the use of α-tocopherol and thus protects our biological systems [3].

Therefore, this study was aimed to investigate the beneficial effect of α-tocopherol administration on Cr(VI) induced histopathological changes in kidney.

MATERIALS AND METHODS

The present experimental study was conducted in the Department of Anatomy, Subharti Medical College, Meerut, Uttar Pradesh, India, for a period of six months from July 2014 to December 2014 after taking the approval from the Institutional Animal Ethics Committee.

Thirty-six albino rats of both sex, aged about 60 days weighing approximately 140±10 gm each were included in this animal model study. All the albino rats were housed in polypropylene cages and were maintained at optimal conditions as per the guidelines of the college committee regarding supervision and control for animal experiments. The animals were kept in a well-lighted and ventilated room and left to acclimatise for one week before the start of experiment. No artificial light was used. These albino rats were given water soaked 200 gm of black gram daily as food and water.

Experimental Protocol

All the albino rats were divided equally into four groups, consisting nine, in each.

Group I: Served as controls and received no drug.

Group II: The rats in this group received Cr(VI) as $K_2Cr_2O_7$ dissolved in sterile distilled water 10 mg/kg body weight single dose orally. The solution was prepared using 100 mg of potassium dichromate dissolved in 10 mL of distilled water. The animals were further divided into three sub-groups (3 in each group) according to numbers of days of drug administration:

- Acute: for one day.
- Subacute: daily for 14 days.
- Chronic: daily for 42 days.

Group III: The animals in this group were also further divided into three subgroups as in group II. They received Cr(VI) as in group II, but along with Cr VI, they also received α-tocopherol 125 mg/kg body weight daily orally.

Group IV: They were maintained as α-tocopherol control and they received α-tocopherol as above daily for 42 days.

Chemicals

Potassium dichromate ($K_2Cr_2O_7$) and α-tocopherol (vitamin E) both were of analytical grade.

Calculation of doses: The criteria for dose selection of α-tocopherol and Cr(VI) was based on a report by Arreola Mendoza L et al., and Biber TU et al., [3,20].

Administration of drug: Both Cr(VI) and α-tocopherol were administered after proper calculation of doses by oral canula fitted into the insulin syringe.

Collection of material: After the completion of administration of the drugs for the estimation of the histopathological changes in kidney, the animals were sacrificed by stroking the dorsal aspect of body. Kidney was carefully separated and immediately two samples of tissues about 1 cm thickness were taken, one from center and another from periphery. Then this was followed by immersion fixation of the kidney tissues in 10% buffered formalin.

Routine processing was carried out for preparation of paraffin blocks. A 5-7 micron thickness sections were cut with Shandon microtome (model-Finesse 325). These were stained with Haematoxylin and Eosin (H&E) stain.

Images were captured with the aid of Motic imaging software. A total of 30 non overlapping fields per slide were examined for each of different histopathological changes.

STATISTICAL ANALYSIS

The comparison between different experimental groups were performed using Statistical Package for Social Science software (SPSS 21.0 software version). The Z-proportion test was used for evaluation of data. A p-value < 0.05 was taken as statistically significant.

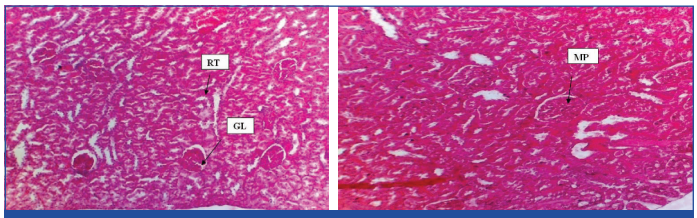
RESULTS

The administration of potassium dichromate in rats did not show any change in water consumption, food consumption or body weight with a mean body weight of 140±10 gm. No mortality was observed in either groups. Also, we did not observe alterations that suggested the presence of untoward effects attributable to α-tocopherol, on its own.

Under light microscopy, the H&E stained section of kidney of all the groups were observed histologically. Similar histopathological findings in both the samples from center and periphery were observed.

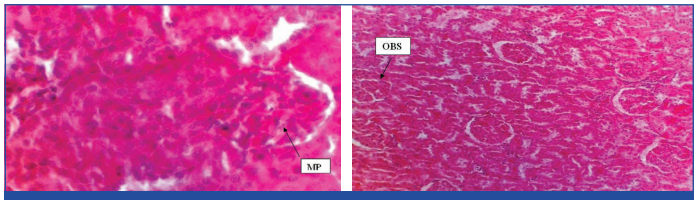
Group I (control): The H&E stained section of kidney of control group showed the normal architecture of renal tissue, without any inflammatory changes [Table/Fig-1].

Group II Cr (VI): The rats of this group received potassium dichromate orally for one day (acute), 14 days (subacute) and 42 days (chronic). Kidney sections of Cr(VI) treated group showed remarkable changes in the histology of kidney like obliteration of bowman's space due to mesangial proliferation in glomeruli in chronic subgroup. Whereas in acute and subacute groups, the similar histopathological findings were seen but the number of affected fields per slide were relatively less as compared to chronic subgroup [Table/Fig-2,3].



[Table/Fig-1]: Photomicrograph of the kidney of control rats showing normal architecture of renal tubules and glomeruli (H&E, X100). GL- Glomerulus, RT- Renal tubules. **[Table/Fig-2]:** Photomicrograph of the kidney of Cr(VI) toxicity groups of rats (chronic) showing obliteration of bowman's space due to mesangial proliferation in glomeruli (H&E, X100). MP- Mesangial proliferation. (Image from left to right)

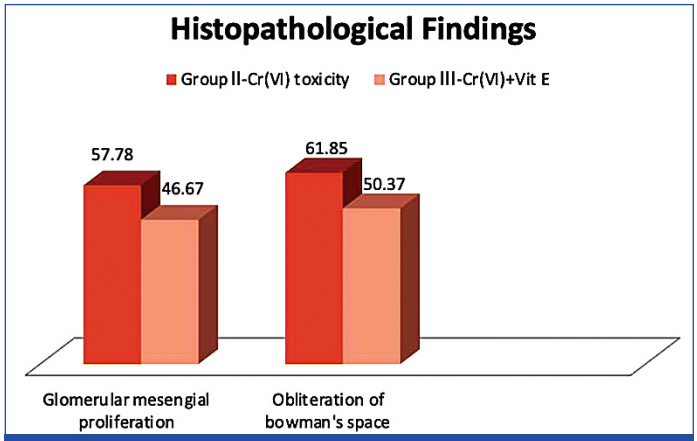
Group III (Cr(VI) + α-tocopherol): In this group, the rats received potassium dichromate along with α-tocopherol orally for one day (acute), 14 days (subacute) and 42 days (chronic). In chronic subgroup, there is presence of mesangial proliferation in glomeruli and obliteration of bowman's space but these pathological changes were reduced as compared to group II. Whereas in other two subgroups, the histopathological findings were similar but the number of affected fields per slide were relatively less as compared to chronic subgroup [Table/Fig-4].



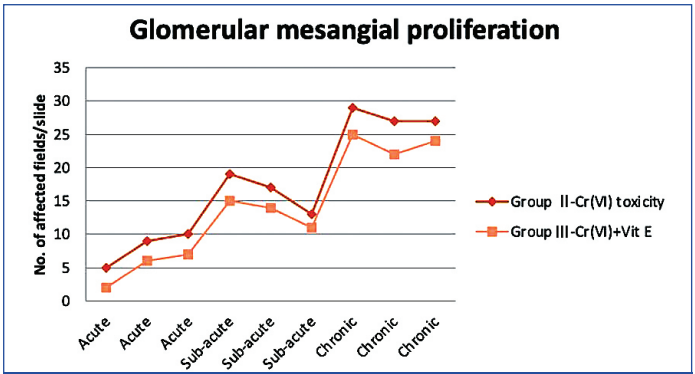
[Table/Fig-3]: Photomicrograph of the kidney of Cr(VI) treated rats (chronic) showing mesangial proliferation in glomeruli (H&E, X400). MP- Mesangial proliferation. **[Table/Fig-4]:** Photomicrograph of the kidney of Cr(VI) + α- tocopherol treated rats (chronic) showing fewer obliterated bowman's space due to mild mesangial proliferation in glomeruli (H&E, X100). OBS- Obliterated bowman's space. (Image from left to right)

Group IV (α-tocopherol control): The histopathological evaluation of only α-tocopherol treated rats showed normal renal architecture.

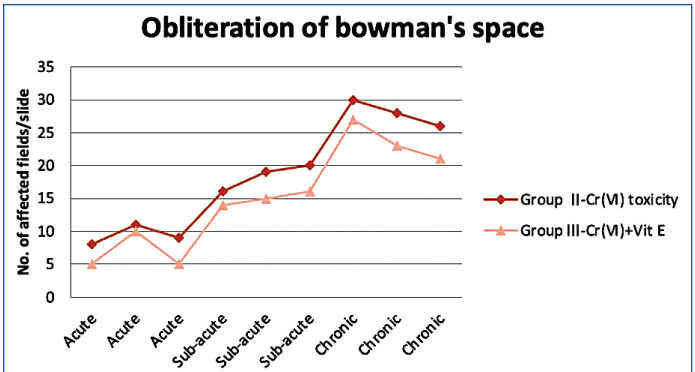
A total of 30 non overlapping fields per slide in group II and group III for histopathological changes were examined. Glomerular mesangial proliferation in group II was found to be 57.78% whereas in group III it was 46.67% with a difference of 11.11 and p-value=0.009. Obliteration of bowman's space in group II was found to be 61.85% whereas in group III it was 50.37 % with a difference of 11.48 and p-value=0.007 [Table/Fig-5-7].



[Table/Fig-5]: Showing comparison of proportion of various histopathological findings between two groups.



[Table/Fig-6]: Showing comparison of glomerular mesangial proliferation observed between two groups.



[Table/Fig-7]: Showing comparison of obliteration of bowman's space observed between two groups.

DISCUSSION

In our environment, heavy metals are widely distributed and some have been attributed to cause physiological, biochemical and histological disorders. There are numerous sources like contaminated air, water, soil and food by which humans can get exposed to these metals [2]. That's why it is important to evaluate the toxic potentials of heavy metals and the risk assessment in human beings.

Varied histopathological changes have been observed in kidney in the present study. In Cr(VI) treated group a general architectural derangement, including mesangial proliferation of glomeruli and obliteration of bowman's space were observed. In another group, where α-tocopherol was given along with Cr(VI) the histopathological changes in kidney were decreased.

The oxidative stress related injury by Cr(VI) may be attributed to the formation of ROS, which causes cellular reduction of Cr(VI) into Cr (III) resulting in oxidative damage of renal tissues and LPO [21]. ROS includes superoxide anion radical (O_2), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH) [22]. An elevated level of H_2O_2 can activate the peroxidation of polyunsaturated fatty acids leading to derangement in cellular structure, function and inactivation of several membrane bound enzymes [23]. This is shown by the remarkable elevation of malondialdehyde (MDA) levels and decrease in antioxidant enzyme activities. Glutathione (GSH), the most abundant non protein thiol endogenous antioxidant antagonises the onset of ROS, while decreased GSH level may hamper the clearance of H_2O_2 leading to increased production of OH, causing oxidative damage. H_2O_2 is also detoxified into water and oxygen by the Catalase activity (CAT) [24]. Superoxide Dismutases (SOD) catalyses the dismutation of (O_2) and formation of H_2O_2 and protects cells from superoxide toxicity [25].

The oxidative stress related injury to kidney may be reduced or slowed down by giving the Vitamin E supplementation, which corrects plasma antioxidant status and mitigates the heart disease which may be associated with renal disease. Vitamin E allows free radicals to abstract a hydrogen atom from the antioxidant molecule rather than from polyunsaturated fatty acids, thus breaking the chain of free radical reactions [26]. So, vitamin E supplementation may prevent free radical damage to the organism from toxic agents. It is inferred that vitamin E is an essential component for the protection of renal tissue against peroxidative damage [27].

Abdel-Rahman GH et al., investigated potassium dichromate induced nephrotoxicity in adult male rabbits and showed structural abnormalities in the renal cortex including glomeruli and tubules. The histopathological changes include shrinkage of the glomeruli, tubular epithelial cells degeneration, tubular swelling and presence of hyaline cast in renal tubular lumens. Lymphocytic infiltration and congestion were also observed [28].

Similarly Balakrishnan R et al., on histopathological examination of kidney in chromium treated group reveled degeneration of tubular epithelial cells, cystic dilatation of tubules, hyaline casts and congestion of blood vessels [15]. The vitamin E co-administered group showed recovery of the histological changes in kidney. Only vitamin E administered group showed normal architecture of kidney.

Aslam M et al., on histopathological examination found normal glomerulus and tubules both in cortical and medullary regions of kidney in control rats [29]. In potassium dichromate treated rats there was severe glomerular and peritubular congestion. In both cortical and medullary section severe invasion of inflammatory cells were seen. In addition to these features necrosis of tubular structure was seen in proximal and distal portions.

Mehany HA et al., studied histopathological picture of kidney showing coagulative necrosis of most of the convoluted tubules at the cortex and the loss of the nuclei in the lining epithelium of the necrotic tubules [30], they concluded that the histopathological changes in kidney were markedly improved by two weeks pretreatment atorvastatin or Vitamin E before dichromate administration.

Saber TM et al., conducted a study in 2015 on the histopathological examination of H&E stained kidney sections of Cr(VI)- treated rats showed severe congestion, haemorrhages, dilated renal tubules, atrophy and atresia of variable regions of the medullary renal tubules with abundant lymphocytic infiltration [31]. Congested glomerular capillaries, hyaline droplet degeneration, shrunken glomerular tufts and congested peritubular capillaries were also visualised. The lesions in the Cr(VI)+ extra virgin olive oil treated rats were remarkably decreased as compared to the Cr(VI)- treated rats similar to the present study.

Limitation(s)

Present study did not focus on electron microscopic evaluation, thus further studies on electron microscopy is needed to demonstrate ultrastructural changes which will enhance our understanding of the light microscopic changes observed by us.

CONCLUSION(S)

The free radicals formed by the chromium compounds in the kidney caused histopathological changes like glomerular mesangial proliferation and obliteration of bowman's space by reducing the antioxidant indices. However, less adverse effects were seen in rats fed with α-tocopherol along with chromium indicating its protective antioxidant property. Thus, the role of α-tocopherol in preserving structural integrity of subcellular organelles by scavenging of free radicals has been proved by the present study. Histomorphometric studies and biochemical analysis can also be done to evaluate the Cr(VI) induced nephrotoxicity.

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