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Protective effect of vitamin E and atorvastatin against potassium dichromate-induced nephrotoxicity in rats

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ABSTRACT

Potassium dichromate, a Cr (VI) compound, is the most toxic form of Cr (VI) and has been demonstrated to induce nephrotoxicity associated with oxidative stress in humans and animals. The wide environmental distribution of Cr lead to an increased interest of its toxicity and biological effects. The present study was designed to investigate the protective effect of vitamin E and atorvastatin against potassium dichromate-induced nephrotoxicity in rats. A single injection of potassium dichromate (15 mg/kg) to rats induced renal tubule damage and an increase in the following markers of renal injury 2 days later; blood urea nitrogen and serum creatinine. In addition, potassium dichromate injection increased the following nitrosative and oxidative stress biomarkers in kidney; malondialdehyde (MDA), total nitrate/nitrite (No_x). This was associated with a significant reduction in kidney glutathione (GSH), metallothionein (MT) contents and superoxide dismutase (SOD) activity. Furthermore inflammatory mediators such as myeloperoxidase (MPO) and tumor necrosis alpha (TNF-α) were increased. Renal damaged was also evidenced by the change in the kidney histopathological picture. Two weeks pre-treatment with vitamin E or atorvastatin before dichromate administration markedly improved its toxicity as indicated by reduction of serum urea and creatinine as well as improvement of kidney histopathological changes. Oxidative stress biomarkers such as renal MDA and nitric oxide contents were also decreased. Kidney superoxide dismutase activity was restored after pre-treatment with vitamin E. Furthermore, atorvastatin significantly reduced TNF-α content and MPO activity while vitamin E reduced TNF-α content. It could be concluded that the ability of vitamin E

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as well as atorvastatin to ameliorate potassium dichromate-induced renal injury was associated with their antioxidant and anti-inflammatory properties.

1. Introduction

Potassium dichromate ($K_2Cr_2O_7$) is a chemical compound widely used in metallurgy, chrome plating, chemical industry, textile manufacture, wood preservation, photography and photoengraving, refractory and stainless steel industries and cooling systems (Barceloux, 1999). The oxidation state and solubility of chromium (Cr) compounds determine their toxicity. In contrast to Cr (III), which is a naturally occurring form and an essential trace element for humans and others mammals, Cr(VI) compounds are highly toxic (Wang et al., 2006). Potassium dichromate is a hexavalent form of Cr and has been demonstrated to induce oxidative stress and carcinogenic in nature (Stohs and Bagchi, 1995; Bagchi et al., 2002a,b).

Vitamin E is a naturally occurring, potent lipid-soluble, chain-breaking antioxidant that scavenges reactive oxygen species and lipid peroxyl radicals both in vitro and vivo (Kir et al., 2005; Arreola-Mendoza et al., 2006). It protects the integrity of membrane by inhibiting lipid peroxidation and augmenting the activity of antioxidant enzymes in the kidney of diabetic rats (Kedziora-Kornatowaska et al., 2003) and is also shown to suppress oxidative stress in rat remnant kidney (Hahn et al., 1999).

Statins may exert lipid independent benefits against renal injury in experimental states of chronic or acute renal function impairment. Also statins, by reducing the synthesis of mevalonate products, inhibit the activation of Rho and Ras guanosine triphosphatases that may influence various signaling pathways involving renal inflammatory, proliferative, and cell-death responses.Therefore, statins exert anti-inflammatory actions in renal tissue. Renal antioxidant effects with consequent endothelial function regulation of renal vasculature following statin treatment may also account for pleiotropic protection against renal injury (Kostapanous et al., 2009).

Therefore, the main goal of this study was to investigate the protective effect of vitamin E and atorvastatin against potassium dichromate induced renal damage.

2. Materials & methods

2.1. Adult male Wistar rats used in this study were randomly divided into 4 groups of eight animals each: The first group received vehicle and served as normal control group; the second group was subjected to a single s.c injection of potassium dichromate (15 mg/kg) to induce renal damage according to the method of Biber et al. (1968). While the third and fourth groups were pretreated with vitamin E (200 mg/kg) or atorvastatin (10 mg/kg) respectively for 14 days before potassium dichromate and continued for 2 more days.

2.2. Forty eight hours after potassium dichromate administration animals were sacrificed by decapitation, blood samples were collected and serum separated for estimation of urea and creatinine. Kidneys were removed and homogenized in ice-cold isotonic saline using (Ultra-Turrax T25, IKA Labortechnik, Germany) for determination of oxidative stress biomarkers and inflammatory mediators. In addition, histological examination of the kidneys was investigated.

2.3. Drugs & chemicals: Vitamin E® capsules (200 mg) was obtained from Farco pharmaceuticals and mixed with sesame oil. Atorvastatin was obtained from E.P.I.C.O pharmaceuticals and suspended in 0.5% carboxy methyl cellulose. Potassium dichromate was purchased from Sisco Qualigens chemicals (Mumbai, India).

2.4. Assay for GSH content was determined in kidney homogenate according to the method of Ahmed et al. (1991).

2.5. Assay for MDA content was carried out according to the method of Uchiyama and Mihara (1978).

2.6. Assay for Kidney (MPO); extraction of MPO depends upon procedures that disrupt the granules and render MPO soluble in aqueous solution. This could be achieved by freezing and rethawing followed by sonication in phosphate buffer pH 6 containing 0.5% hexadecyl trimethyl ammonium bromide HTAB (Bradley et al., 1982).

2.7. Assay for TNF-α was carried out using rat TNF-α immunoassay kit (eBioscience, USA).

2.8. Assay for Kidney total Nitrate/Nitrite Content (NO_x) was determined as an index of nitric oxide (NO) content in renal homogenate according to the method described by Miranda et al. (2001).

2.9. Assay for metallothionein (MT) content in kidney was assayed according to the method of Viarengo et al. (1997) modified by Petrovic et al. (2001). The metallothionein content was evaluated using the colorimetric method with Ellman's reagent.

3. Statistical analysis

Results are expressed as mean ± standard error (S.E.). The statistical significance of differences between the experimental groups was calculated by ANOVA followed by Tukey–Kramer tests. Analyses were performed using the statistical software Graph Pad InStat. Results were considered significant when $P < 0.05$.

4. Results

As in Fig. 1(a & b), subcutaneous injection of a single dose of potassium dichromate caused a significant elevation in serum urea and creatinine. It was shown that vitamin E or atorvastatin pre-treatment caused a significant reduction of the elevated serum urea and creatinine levels.

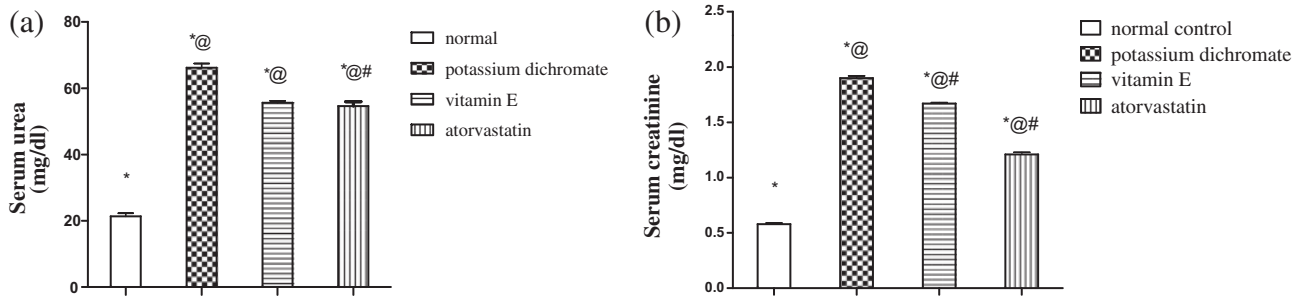

Fig. 1 – Effect of pre-treatment with vitamin E or atorvastatin on serum urea (a) and creatinine (b) levels following potassium dichromate-induced nephrotoxicity in rats. Each value represents mean ± S.E of the mean. * Significantly different from normal control group at $p < 0.05$. @ Significantly different from potassium dichromate group at $p < 0.05$. # Significantly different from vitamin E treated group at $p < 0.05$.

Fig. 2 shows the significant elevation of renal MDA content following potassium dichromate administration. Pre-treatment with vitamin E as well as atorvastatin significantly decreased the elevated renal MDA content.

Fig. 3 shows that the renal SOD activity was significantly reduced following potassium dichromate administration and the pre-treatment with vitamin E significantly restored the renal SOD activity.

The reduction in renal GSH and MT content following potassium dichromate administration is shown in Table 1. Neither vitamin E nor atorvastatin significantly affected the GSH and MT contents, although pre-treatment with vitamin E normalized GSH.

Table 2 shows that potassium dichromate significantly increased renal MPO activity as well as TNF-α and NO_x contents. Atorvastatin pre-treatment significantly reduced MPO activity TNF-α and NO_x contents. Pre-treatment with Vitamin E significantly reduced the elevated TNF-α and NO_x contents.

Fig. 4 shows that sections of normal kidneys (a) showing the normal architecture of renal tissue, being composed of a number of glomeruli embedded among a great number of different tubules. Rats subjected to potassium dichromate (b) 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. Sections of kidneys of rats (c) pre-treated with vitamin E and subjected to potassium dichromate showing mild degeneration in the lining epithelium of some tubules of the cortex. Sections of kidneys of rats (d) pretreated with atorvastatin and subjected to potassium dichromate showing mild focal inflammatory cells infiltration and focal hemorrhage in between the tubules at the cortex.

5. Discussion

The kidney is the main route of Cr excretion, it has been reported that acute exposure to potassium dichromate in rats induced an increase in kidney Chromium content (Travacio et al., 2001). Although chromium itself does not directly generate free radicals, it indirectly generates various radicals such as superoxide, nitrogen species like peroxynitrite, nitric oxide and hydroxyl causing damage consistent with oxidative stress (Pritchard et al., 2000).

Results of the present study showed marked increase in serum creatinine and urea levels following potassium dichromate administration. In addition potassium dichromate induced toxic injuries to the renal tubules and loss of functional integrity in the kidney as seen by histopathologial

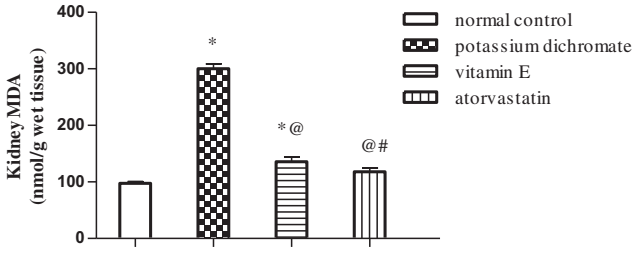

Fig. 2 – Effect of pre-treatment with vitamin E or atorvastatin on kidney malondialdehyde (MDA) content following potassium dichromate-induced nephrotoxicity in rats. Each value represents mean ± S.E of the mean. * Significantly different from normal control group at $p < 0.05$. @ Significantly different from potassium dichromate group at $p < 0.05$. # Significantly different from vitamin E treated group at $p < 0.05$.

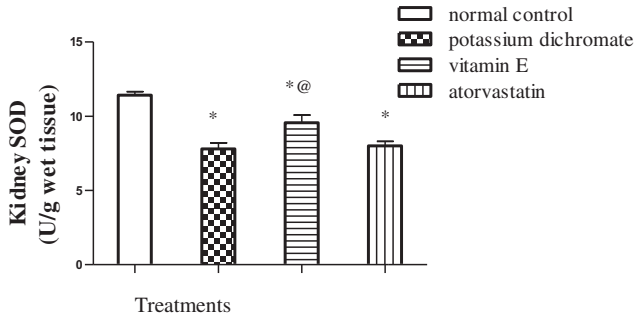

Fig. 3 – Effect of pre-treatment with vitamin E or atorvastatin on kidney superoxide dismutase (SOD) content following potassium dichromate-induced nephrotoxicity in rats. Each value represents mean ± S.E of the mean. * Significantly different from normal control group at $p < 0.05$. @ Significantly different from potassium dichromate group at $p < 0.05$. # Significantly different from vitamin E treated group at $p < 0.05$.

Table 1 – Effect of pre-treatment with vitamin E or atorvastatin on kidney glutathione (GSH) and metallothionein (MT) contents following potassium dichromate-induced nephrotoxicity in rats.

Treatments and parameters	GSH (μg/g wet tissue)	MT (μg/g wet tissue)
Normal control	12.20 ± 0.25	101.95 ± 6.20
Pot. dichromate	10.9 ± 0.24*	58.20 ± 5.44*
Vitamin E + Pot. dichromate	11.92 ± 0.23	45.57 ± 2.92*
Atorvastatin + Pot. Dichromate	10.85 ± 0.38*	47.37 ± 3.00*

Each value represents mean ± S.E of the mean.
*Significantly different from normal control value at $p < 0.05$.

examination of the current study and as reported before (Arreola-Mendoza et al., 2006). Previous study showed that exposure to Cr (VI) compounds can lead to nephrotoxicity in humans and experimental animals (Fatima et al., 2005). The role of oxidative stress in dichromate-induced kidney damage has been supported by the present work and previous studies (Pedraza-Chaverri et al., 2008; Yam-canul et al., 2008). Inside the cell, Cr (VI) is reduced to Cr (III). This reduction process generates reactive oxygen species (ROS) and induces soft tissues damage such as liver, pancreas, cerebellum and kidney (Bagchi et al., 2002a,b; Fatima et al., 2005). Large amounts of ROS generated by this process can bring on injury to cellular proteins, lipids, and DNA leading to oxidative stress (Nordbeg and Arner, 2001). The observed increase in MDA and NO is a good evidence for this oxidative stress. Potassium dichromate may lead to induction of inducible nitric oxide synthase, resulting in an increased production of NO and formation of toxic peroxynitrite (Coppo and Amore, 2000). Moreover an enhancement of NO was reported by Bagchi et al. (1995) in peritoneal cells from sodium dichromate-administered rats. Furthermore potassium dichromate administration resulted in the reduction of renal GSH content as compared to normal control group which is in accordance with results obtained before by Khan et al. (2010) and Molina-Jijón et al. (2012).

Glutathione is normally present in millimolar concentrations in cells and is known to protect the cellular system against the toxic effects of lipid peroxidation. It is very important in maintaining cellular redox status (Rao and Shaha, 2001) and its depletion is considered as a marker of oxidative stress (Lu, 1999).

In the present study, administration of potassium dichromate significantly decreased the renal metallothionein (MT) level. Metallothionein can't bind Cr, but by scavenging ROS (Thornally, 1985) it may act as a protective factor against Cr (VI)-induced DNA lesions, reducing Cr (VI) directly to Cr (III). Kimura et al. (2008) reported that Cr (VI) inhibited the ability of MT to trans-activate its gene in response to zinc and that potassium dichromate interfered with the capacity of MT to form a co-activator complex containing histone acetyl transferase and recruiting RNA polymerase II to the promoter.

In addition administration of dichromate significantly reduced renal SOD activity. The decreased SOD activity may lead to massive production of superoxide anion. The production of such anions overrides enzymatic activity and leads to a fall in its concentration in renal tissue (Srinivasan et al., 2008). Pedraza-chaverri et al. (2005) indicated that most of the antioxidant enzymes become inactive after potassium dichromate exposure either due to the direct binding of heavy metals to enzyme active site if it contains SH group or to the displacement of metal co-factors from active sites.

Another explanation of renal injury-induced by dichromate is mediation of inflammatory process as seen by increased pro-inflammatory cytokine renal TNF-α content and MPO. This was shown previously by Wang et al. (2010) who reported that hexavalent chromium could increase ROS formation, activate the Akt, NF-kB, and MAPK pathways as well as increase the production of cytokines, including TNF-α and IL-1α. Furthermore, Gueniche et al. (1994) showed that potassium dichromate could stimulate the release of cytokines, such as TNF-α in normal human keratinocytes.

Pre-treatment with vitamin E significantly reduced the elevated serum creatinine and urea levels and improved kidney histopathological picture. These results prove that obtained before by Khan et al. (2010) who showed the protective role of tocotrienol against potassium dichromate-induced nephrotoxicity. Moreover, Kagan et al. (1989) reported that one of the ways in which α-tochopherol is believed to stabilize membranes is to form a complex with the membrane lipids components that have a tendency to destabilize the bilayer structure thereby countering their effects and rendering the membrane more stable as also supported by the observed reduction of MDA and nitric oxide as well as increased glutathione.

In addition, Halliwell and Gutteridge (2002) suggested that treatment with α-tocopherol averted oxidative damage, probably through its capacity to quickly and efficiently scavenge lipid peroxide radicals before they attack the membrane lipids. This ability might be related to the fact that lipid peroxyl radicals react more rapidly (by four orders of magnitude)

Table 2 – Effect of pre-treatment with vitamin E or atorvastatin on kidney myeloperoxide (MPO) activity, TNF-α and NO_x contents following potassium dichromate-induced nephrotoxicity in rats.

Treatments and parameters	MPO (U/g wet tissue)	TNF-α (pg/gwet tissue)	NO_x (μmol/g wet tissue)
Normal control	0.44 ± 0.02	1484.24 ± 40.33	140.52 ± 13.38
Pot. dichromate	1.41 ± 0.05*	2187.46 ± 90.21*	269.74 ± 8.98*
Vitamin E + Pot. dichromate	1.25 ± 0.04*	1744.79 ± 51.84@	202.04 ± 14.48*@
Atorvastatin + Pot. dichromate	1.15 ± 0.10*@	1406.24 ± 69.87@	166.44 ± 14.64@

Each value represents mean ± S.E of the mean.
*Significantly different from normal control value at $p < 0.05$.
@ Significantly different from potassium dichromate group at $p < 0.05$.

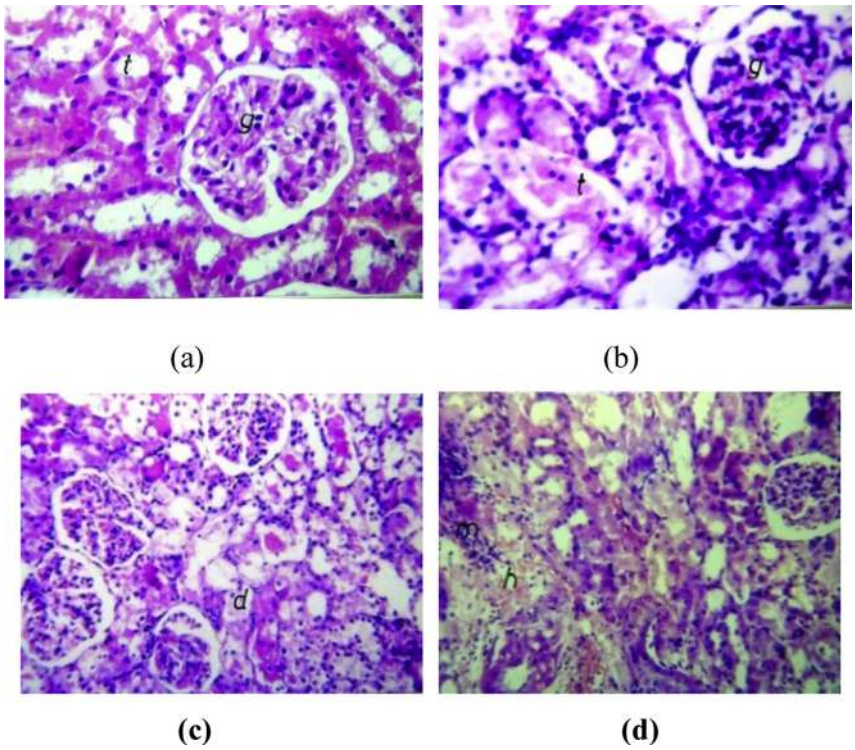

Fig. 4 – (a) normal rat showing the normal architecture of renal tissue. (b) rats subjected to potassium dichromate showing coagulative necrosis of most of the convoluted tubules at the cortex and the loss of the nuclei in the lining epithelium of the necrotic tubules. Sections of kidneys of rats(c) pretreated with vitamin E and subjected to potassium dichromat showing mild degeneration in the lining epithelium of some tubules of the cortex. (d) pretreated with atorvastatin and subjected to potassium dichromate showing mild focal inflammatory cells infiltration and focal hemorrhage in between the tubules at the cortex.

with α-tocopherol, than with membrane lipids. Furthermore pre-treatment with vitamin E significantly increased the renal SOD activity.

Pre-treatment with vitamin E significantly reduced dichromate-induced elevation in TNF-α. Vitamin E, in addition to its direct antioxidant effects, may offer indirect protection by decreasing neutrophil recruitment (Blesa et al., 2003; Kolleck et al., 2002).

Moreover, Azzi et al. (2002) reported that α-tocopherol inhibits protein kinase C in various cell types, with the consequent inhibition of platelet aggregation, nitric oxide production in endothelial cells, and superoxide radical generation by neutrophils and macrophages.

In the current study, pre-treatment with atorvastatin (ATO) reduced the elevated serum creatinine and urea levels induced by potassium dichromate and improved kidney histopathological picture. Similarly Ozbek et al., (2009) reported a protective effect of atorvastatin in gentamicin-induced nephrotoxicity. Cuzzocrez et al. (2002) referred that protective role of atorvastatin could be attributed to its antioxidant effect because it has been found that ROS may be involved in the impairment of glomerular filtration rate. Antioxidant effect was also shown in this study where MDA and nitric oxide were reduced.

Statins have been shown to reduce lipoprotein oxidation and ameliorate free radical injury, and ATO possesses significant antioxidant activity against OH and peroxyl radicals. Furthermore, metabolites of ATO reduce lipoprotein oxidation in a number of oxidative systems (Aviram et al., 1989). Moreover, Iseri et al. (2007) reported that simvastatin attenuated cisplatin-induced kidney damage via prevention of lipid peroxidation. Previous studies demonstrated that statins reduced ROS and superoxide anion renal production either through down-regulation of NADPH oxidase activity or by a decrease in the renal endothelial expression of inducible NOS (Kostapanos et al., 2008; Yagiet al., 2008).

In the current study, pre-treatment with ATO significantly reduced the elevated renal TNFα and MPO activity.

Inhibition of increased MPO activity may result in decreased iNOS over expression and consequently lesser generation of reactive oxygen and nitrogen species (Cuzzocrea et al., 2000).

There is emerging evidence to suggest that statins exert anti-inflammatory effects by blocking the infiltration of inflammatory cells and down regulating the expression of inflammatory mediators, such as IL-6 and C-reactive protein (Gueler et al., 2002; Sharyo et al., 2008).

Previous in vitro and in vivo results (Lopez et al., 2000; Diomede et al., 2001) indicating that statins suppress the synthesis of inflammatory mediators, such as TNF-α. Likewise Mira et al. (2003) observed that simvastatin and cervistatin decreased TNF-α and MPO through mevalonate-independent pathways, but possibly by inhibiting the ERK pathway.

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