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Research Article

AMELIORATIVE ROLE OF VITAMIN E AGAINST HEXAVALENT CHROMIUM INDUCED HEPATO–NEPHROTOXICITY IN LABORATORY CHICKS: A HISTOPATHOLOGICAL STUDY

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ABSTRACT

Chromium is a widespread environmental waste. It is an industrial contaminant with teratogenic, mutagenic and carcinogenic effects on animals and human. Present study was carried out to evaluate the potential protective effect of vitamin E on the hepatotoxicity and nephrotoxicity generated by potassium dichromate ($K_2Cr_2O_7$) in laboratory chicks. The histopathological evaluation of liver and kidney showed severe changes in chicks treated with $K_2Cr_2O_7$. Liver of the $K_2Cr_2O_7$ treated group showed major histological alterations, such as necrosis of hepatocytes, dilatation of sinusoids with congestion of blood vessels and hemorrhage. In chromium intoxicated chicks, congestion and hemorrhages in renal tissues, hemorrhages in kidney parenchyma, glomeruli segmentation and swelling of glomeruli with infiltration of leucocytes (Glomerulitis) were seen. Administration of vitamin E protects the liver and kidney damaged by $K_2Cr_2O_7$ as evidenced by appearance of normal histological structures, although hemorrhage was also noticed. Vitamin E treatment showed significant improvement in the histopathological picture. It could be concluded that potassium dichromate is potent hepatotoxic and nephrotoxic. Vitamin E has a potential protective effect to reverse the toxicity of $K_2Cr_2O_7$ and has the ability to improve the hepatic and renal tissue damage associated with $K_2Cr_2O_7$ intoxication.

Keywords: Chromium, Hepatotoxicity, Nephrotoxicity, Vitamin E, Intoxication

1. INTRODUCTION

Chromium (Cr) is a naturally occurring heavy metal commonly found in the environment in two valence states: trivalent Cr(III) and hexavalent Cr(VI). It is widely used in numerous industrial processes, and thus is a contaminant of many environmental systems [1]. It is commonly used in various industries (e.g., steel, alloy, cast iron, chrome plating, paints, leather tanning, photography, and metal finishes). However, Cr(VI) compounds have been reported to be more toxic and carcinogenic than those of Cr(III) because the former can pass through cell membranes more easily than the latter [2].

Once inside the cell, Cr(VI) is reduced to its lower oxidation states, Cr(V) and Cr(IV), and then to Cr(III) by low-molecular weight molecules, enzymatic reductants, and non-enzymatic reductants [3]. These reactive chromium intermediates are capable of generating a whole spectrum of reactive oxygen species (ROS), which is an important characteristic of Cr(VI) metabolism [4]. An excessive quantity of ROS that is generated by these reactions can cause injury to cellular proteins, lipids, and DNA, leading to a state known as oxidative stress [5-7]. Therefore, one of the most important damages caused by extraneous Cr(VI) is the massive ROS production during Cr(VI) reduction in the cell.

However, the liver is the major organ responsible for metabolism, detoxification, and secretary functions in the body. Hence, it regulates various important metabolic functions in mammalian systems [6]. However, Cr(VI) has been reported to cause hepato - nephrotoxicity in humans and laboratory animals primarily through an oxidative stress-mediated mechanism [6, 8, 9]. Considering the high sensitivity of the liver to Cr(VI) related insult, preventive intervention is a major concern. Heavy metals are nephrotoxic and xenobiotic that may lead to acute tubular necrosis, loss of brush border [10,11] .The hexavalent chromium compounds are carcinogens, corrosives, delayed contact sensitizers the kidney as a primary target organ [12].

Vitamin E is an important component in human diet and *considered the* most effective liposoluble antioxidant found in the biological system. It is composed of various subfamilies of which tocopherols and tocotrienols are the most studied.

The structural difference between the two subfamilies is that tocotrienols possess three double bonds in their isoprenoid side chain and this structural difference result in difference in their efficacy and potency as antioxidants [13]. Vitamin E is known to have been proven beneficial in some disease processes. It protects the body's biological systems [14] by preventing lipid peroxidation [15].

Vitamin E is also known as a protective antioxidant in progressive renal failure. Dietary vitamin E supplements which maximize the plasma level may be beneficial in slowing progressive kidney diseases that are significantly accelerated by oxidative stress. Many researchers reported protective effects of antioxidant of vitamin E family against metal induced adverse effects in man and laboratory animals [16, 17]. Vitamin E has received wide attention due to its reported hepatoprotective effects in animals, which is primarily due to its ability to attenuate the induced oxidative stress in various tissues by reducing MDA levels; restoring the levels of GSH, SOD, and CAT and the recovery of impaired hepatic cells [18].

Because of the health problems induced by many environmental pollutants, much effort has been expended in evaluating the relative antioxidant potency of vitamin E [19-22]. Therefore, the present study has been designed to investigate the possibilities that the administration of vitamin E would have a beneficial effect against Cr-induced hepatic and renal injuries and hepatic and renal structural changes in chicks.

The aim of the present work was to study the protective role of vitamin E against toxic effects of hexavalent chromium in the form of potassium dichromate ($K_2Cr_2O_7$) on liver and kidney as evidenced by histopathological pictures. In the present study we hypothesize that vitamin E acts as an antioxidant and may play an important role to reduce chromium-induced toxicity in tissues. Therefore, present study has been expected to enhance our understanding of harmful effects of chromium mediated toxicity on organs like liver and kidney and also design a suitable antioxidant therapy against hexavalent chromium as well as other heavy metals.

2. MATERIALS AND METHODS

2.1. Animals

The experiments were carried on Domestic chicks-Croiler Chabro (*Gallus gallus domesticus*). Newly hatched chicks were purchased from the Uttarakhand Village Poultry Project (State Govt. Poultry Farm), Bin, Pithoragarh (Uttarakhand). Selected all chicks were maintained and acclimatized according to the laboratory condition. The animals were housed in battery cages under laboratory conditions at existing room temperature and relative humidity. They were fed on commercial food (Starter, Grower and Finisher) purchased from the local market and tap water *ad libitum*. Healthy male and female chicks (approximately 2-3 weeks old, body weight 100±20 gm) were used in present study. All protocols were approved by the Institutional Animal Ethics Committee (IAEC), Department of Pharmaceutical Science, Bhimtal, Kumaun University, Nainital and the member secretary, CPCSEA, Ministry of Environment, Forest and Climate Change, Government of India (Protocol No.-KUDOPS/89). The animals were kept under standard conditions throughout the experiment to reduce the error. Minimum number of animals was used to obtain reliable results.

2.2. Chemicals

Vitamin E (α-tocopherol) was purchased from Sigma Chemical Co. (St. Louis, Mo, USA). Potassium dichromate ($K_2Cr_2O_7$) was procured from Glaxo (India). Haematoxylin and Eoisin were purchased from S. Merck, Mumbai. All the reagents and chemicals used in this study were of analytical grade and highest purity procured from standard commercial sources in India.

2.3. Experimental Treatments

The selected chicks were divided into three groups (A, B and C) randomly, each containing at least 6 chicks. Chicks of group A were administered with sublethal dose of potassium dichromate ($K_2Cr_2O_7$) (5 mg/100 gm body weight) by gavage on each alternate day for 30 days. Chicks of group B were treated with potassium dichromate ($K_2Cr_2O_7$) as chicks of group A but also administered with vitamin-E (intramuscularly) (0.5 IU/100 gm body weight) on each alternate day for 30 days. Chicks of Group C were administered with saline only to serve as purely control.

2.4. Histopathological Studies

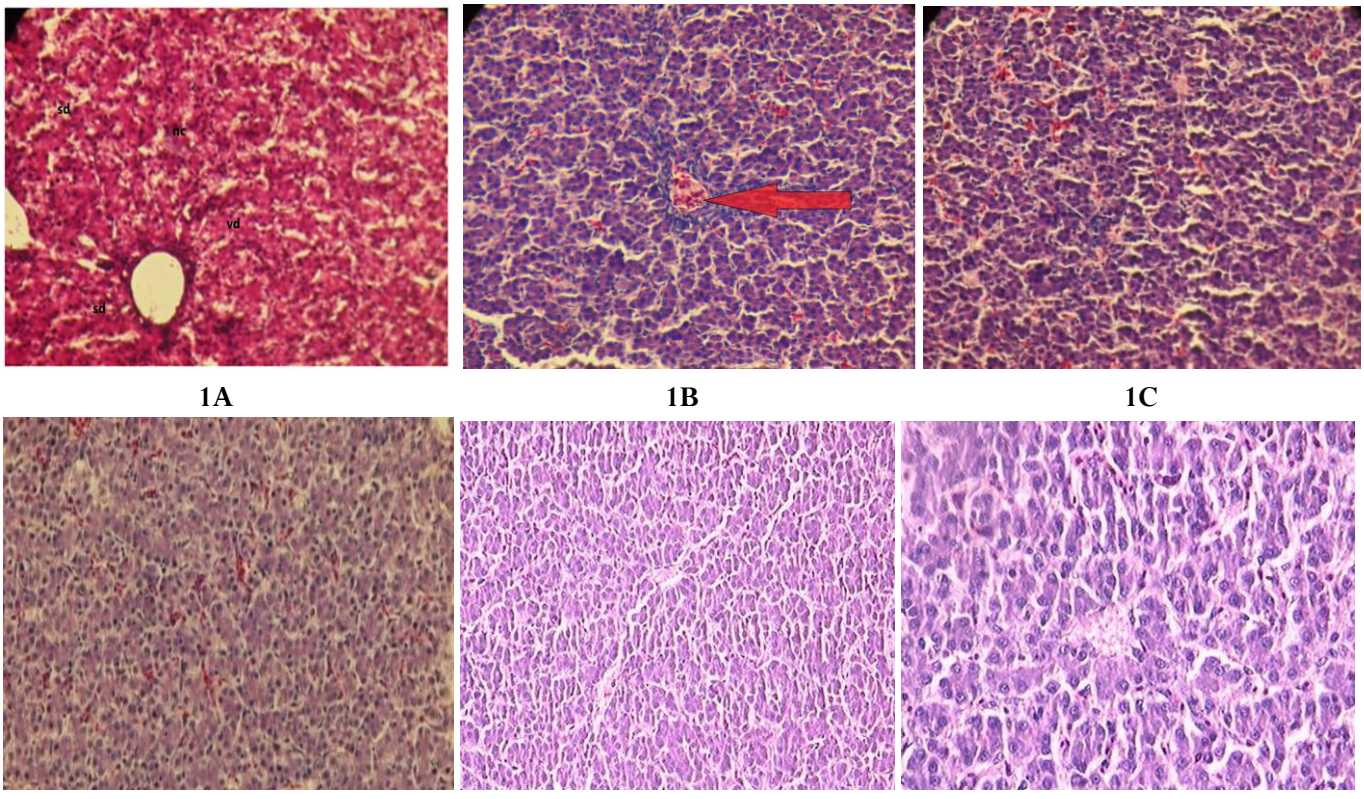
After 30 days of treatment period, chicks from each group were anesthetized with diethyl ether early in the morning and scarified. Liver and kidney were removed carefully and immediately prepared for histopathological examination [23]. The specimen labeled according to their treatment group and fixed in 10% neutral buffered formalin solution for 20-24 hours. Fixed tissues were

processed after washing in running water and preserved in 70% ethanol, dehydrated in a series of graded concentrations of ethyl alcohol to remove the water and formalin from the tissue and cleared in xylene to remove the alcohol and allow infiltration with paraffin wax. Selected tissues were embedded in paraffin waxes (melted paraffin at 55-60^{0}C), casting and cutting at 4-5 µm in thickness using a microtome. These sections were placed on top of glass slides, routinely stained with histochemical stain i.e. haematoxylin and eosin (H and E) to be examined and observed with light microscope.

3. RESULTS AND DISCUSSION

The haematoxylin and eosin (H&E) stained sections of liver and kidney of all the groups were seen under light microscope for histopathological observation.

Prepared slides were examined to observe the histopathological changes in each group. Histopathological examination of the liver and kidney specimens showed severe alterations in chicks exposed to potassium dichromate (Group A). Histopathological findings of liver tissue showed vacuolar degeneration, necrosis and sinusoidal dilatation in the liver of chromium treated chicks (Fig. 1 A) compared to the normal structure of control group (Group C) (Fig. 1 E and F). Congestion in veins was also seen (Fig. 1 B). Recovery from histological injury was observed in vitamin E administered Group B chicks, with normal histoarchitecture of liver tissues, although hemorrhage was still found (Fig. 1 C and D).



1A 1B 1C

1D 1E 1F

Fig. 1(A – F): Photomicrograph of the liver

Fig.1 (A and B) – Liver of Cr intoxicated chicks (H&E, 40X).
Fig 1(C and D) – Liver of Cr + vitamin E treated chicks (H&E, 40X).
Fig 1(E and F) – Liver of control group (H&E, 100X).

Histopathology of kidney sections showed severe alternation of renal tissues in chromium treated chicks when compared with those from the control chicks. Congestion and hemorrhages in renal tissues (Fig. 2 A), congestion along with hemorrhages in kidney parenchyma (Fig. 2 B), glomeruli segmentation (Fig. 2 C) and swelling of glomeruli with infiltration of leucocytes (Glomerulitis) (Fig. 2 D) were seen in chromium intoxicated chicks (Group A). Histopathological findings of kidney sections of Cr

administered and treated with vitamin E group (Group B) of chicks maintained normal histoarchitecture of renal tissues showing recovery from vitamin E treatment, although hemorrhages in kidney parenchyma was also observed (Fig. 2 E).

Recovery was also seen in glomeruli segmentation (Fig. 2 F). Histopathological picture of kidney of control chicks (Group C) showed normal tubules and normal histoarchitecture of renal tissues (Fig. 2 G and H).

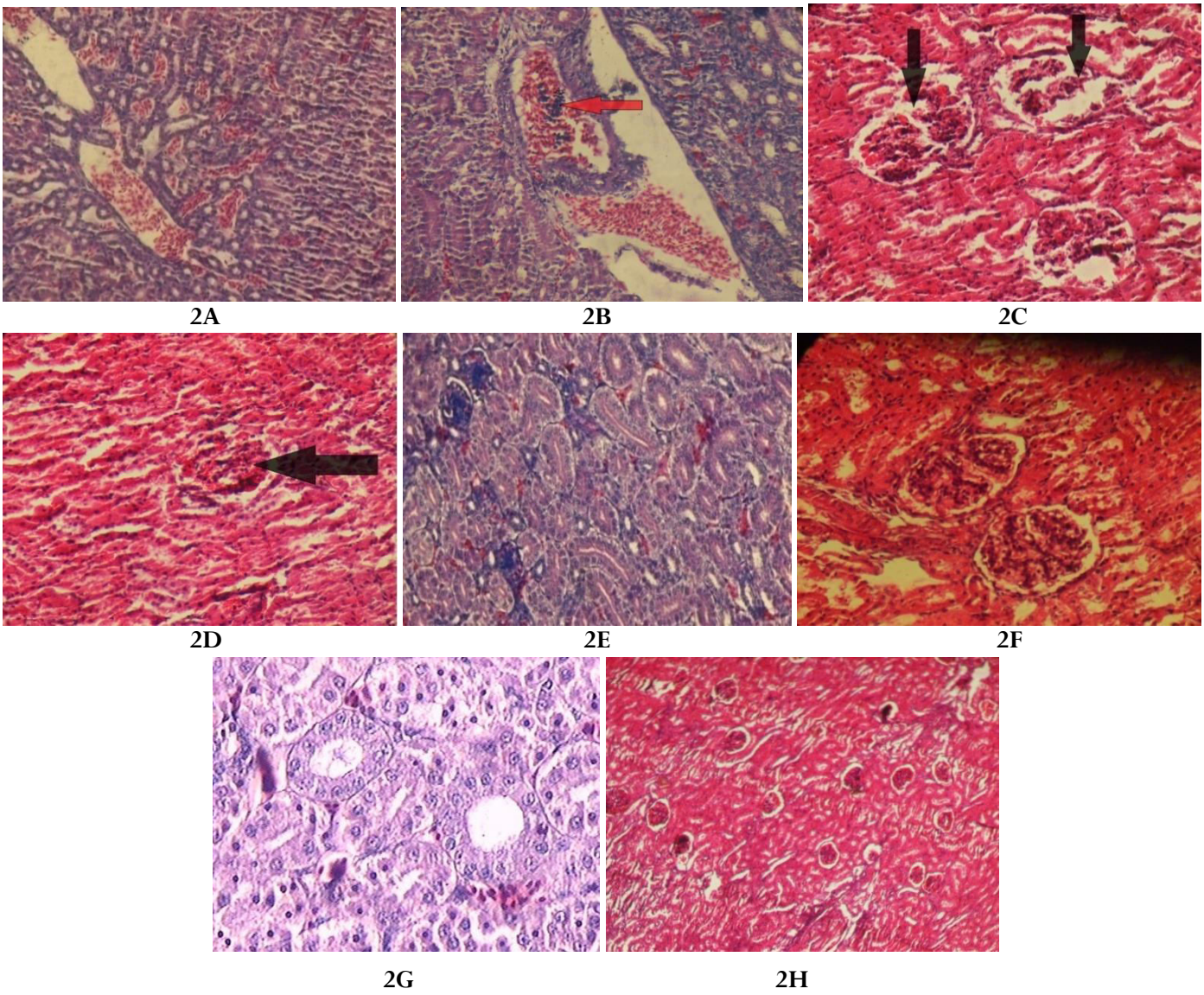


Fig. 2 (A-H): Photomicrograph of the kidney

Fig 2(A - D) - Kidney of Cr intoxicated (H&E, 40X).
Fig 2(E and F) – Kidney of Cr + vitamin E treated chicks (H&E, 40X).
Fig 2(G and H) – Kidney of control group (H&E, 100X and 40X respectively).

It is well known that heavy metals are widely distributed in environment and some of them can cause physiological, biochemical and histological disorders. Humans are exposed to these metals from numerous sources, including contaminated air, water, soil and food. Therefore, the evaluation of toxic potentials of metals is important for the risk assessment of human beings ordinarily exposed to these substances. Different scientific studies indicated that the degree of toxic manifestation of different metals depends on dose, duration, route of administration and other physiological factors, especially nutrition [24]. Among the toxic heavy metals, Cr(VI) compounds are widely recognized as human carcinogens [25]. From the epidemiological

studies, it is suggestive that hexavalent chromium causes increased risk of bone, prostate lymphomas etc. reflecting the ability of hexavalent chromium to penetrate all tissues in the body [26]. Due to their extensive use in the industry, there is a need to investigate their combined toxicity in organ system and mitigative role of vitamins on their toxicity. Previous studies have shown that dichromate exposure increases the concentration of ROS [27] and provokes oxidative damage in hepatocytes [28] and kidney [29].

Administration of Cr(VI) resulted in oxidative stress in the liver and kidney that was reflected by altered histoarchitecture of liver and kidney. The liver of Cr-intoxicated chicks showed degenerative changes and dilatation of sinusoids with congestion of blood vessels and hemorrhage, and necrosis of hepatocytes. Kidney sections of Cr-intoxicated chicks revealed congestion and hemorrhages in renal tissues, congestion along with hemorrhages in kidney parenchyma, glomeruli segmentation, and swelling of glomeruli with infiltration of leucocytes. Severe histological changes in the liver and kidney of Cr treated rats were earlier reported by Acharya et al [30] and Da Silva et al [31] The present work demonstrated that chicks chronically intoxicated by potassium dichromate display a pronounced impairment in liver and kidney which is confirmed by the histopathological alteration.

Liver is known as a toxic target organ of Cr(VI) and other heavy metals, and liver also aids in the metabolism and detoxification of heavy metals and other foreign substance [32,33]. In present study distorted hepatic architecture is seen in Cr-intoxicated chicks. Previous studies of O'Brien TJ [34] have shown that dichromate exposure increases the concentration of reactive oxygen species, which is reflected by damage and distortion of hepatocytes. Pattola et al [35] stated that increase concentration of reactive oxygen species (ROS) lead to oxidative damage of hepatocytes, reflected by altered histoarchitecture changes and dilatation of sinusoids. In present study also damage of liver cells occurs, with dilatation of sinusoids, congestion of blood vessels and hemorrhage, and necrosis and distortion of hepatocytes which further leads to degenerative changes. In various studies, it was suggested that reactive oxygen species (ROS) are involved in Cr(VI)-induced cell injury [4, 36, 37]. The histopathological changes in a liver treated with potassium dichromate ($K_2Cr_2O_7$) may be due to the formation of highly reactive radicals and subsequent lipid peroxidation induced by $K_2Cr_2O_7$ [38]. The accumulated hydroperoxidase can cause cytotoxicity which is associated with the peroxidation of membrane phospholipids by lipid hydroperoxidase, the basis of cellular damage. Ercal et al [39] reported enhancement of lipid peroxidation in rat livers after heavy metal poisoning with mercury, molybdenum, copper, chromium, and manganese. Moreover, Bagchi et al [1] showed that chromium (VI) induce increase in hepatic mitochondrial and microsomal lipid peroxidation.

Considering the adverse effect of Cr(VI) toxicity on liver tissues, preventive intervention is of major concern. Previous studies have shown that antioxidants supplement could protect the liver tissues from oxidation and prevent DNA damage under various toxic conditions [9]. Among these chemical agents is vitamin E, which has drawn wide attention due to its reported hepatoprotective property against metal-induced toxicity. In this study it was seen that when vitamin E was given in chromium intoxicated chicks, there was reversal of histological alteration in liver cell which is similar to findings of Da Silva et al [31]. Such protective effects may be related to the antioxidant potential of vitamin E.

Although in present study, hemorrhages were also observed in liver tissues of Cr and vitamin E simultaneously administered chicks. Sodergren et al [40] concluded that both non-enzymatic and enzymatic lipid peroxidations during experimental hepatic oxidative injury were suppressed by dietary vitamin E supplementation in rats. In addition, the protective effect of vitamin E on Cr VI-induced cytotoxicity and lipid peroxidation in primary cultures of rat hepatocytes was investigated by Susa et al [3] where pretreatment of primary cultures of rat hepatocytes with α-tocopherol succinate for 20 h prior to exposure to $K_2Cr_2O_7$ resulted in a marked decrease of Cr(VI) induced cytotoxicity.

Soudani et al [9] had shown that antioxidant supplement could protect the chromium induced liver tissue damage which is in present study was shown by supplementation of vitamin E along with chromium, where the reversal of the distorted changes of the liver cell occurred to large extent. Bursell and King [41] in his study proved that α-tocopherol, is a chain breaking antioxidant exist in cell membrane and suppresses the chain reaction of lipid peroxidation and promotes the scavenger antioxidant enzyme and helps in reversal of histological alteration. In present study too liver cells treated with vitamin E along with chromium ($K_2Cr_2O_7$) reverse the altered histological changes of liver cells. Hence vitamin E

proved to be hepatoprotective against chromium induced toxicity.

The kidney is the main route of Cr excretion, and it has been reported that acute exposure to potassium dichromate in rats induced an increase in kidney Chromium content [42]. Altuough 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 [43]. Result of the present study showed potassium dichromate induced toxic injuries to the renal tubules, congestion and hemorrhages in renal tissues, congestion along with hemorrhages in kidney parenchyma, glomeruli segmentation, and swelling of glomeruli with infiltration of leucocytes (Glomerulitis) in the kidney as seen by histopathological examination and as reported before [44]. Previous study showed that exposure to Cr(VI) compounds can lead to nephrotoxicity in humans and experimental animals [45]. The role of oxidative stress in dichromate-induced kidney damage has been supported by the present work and previous studies [46, 47]. 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 [45, 48, 49]. Large amounts of ROS generated by this process can bring on injury to cellular proteins, lipids, and DNA leading to oxidative stress [50].

Administration of vitamin E along with potassium dichromate showed improvement in kidney histopathological picture. Khan et al [51] also showed the protective role of tocotrienol against potassium dichromate-induced nephrotoxicity. Halliwell and Gutteridge [52] 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) with α-tocopherol, than with membrane lipids.

Vitamin E supplementation may be beneficial in reducing and slowing progressive kidney diseases that are significantly accelerated by oxidative stress. Vitamin E therapy may also be effective in reducing cardiovascular disease associated with chronic renal failure and the uremia state. Vitamin E therapy is also considered as a mean of correcting plasma antioxidant status and attenuating the cardiovascular disease that accompanies kidney failure. 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, the resulting antioxidant radicals being a relatively unreactive species [53]. In many studies vitamin E neutralizes lipid peroxidation and unsaturated membrane lipids because of its oxygen scavenging effect [54, 55] Vitamin E is an essential component of the kidney for protection of this tissue against peroxidative damage [56].

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