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## Research Article

# Ameliorating Effect of Vitamin C Against Potassium Dichromate Induced Oxidative Stress and Inflammatory Response in Rats

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## Abstract

**Background and Objective:** Potassium dichromate ( $K_2Cr_2O_7$ ) is an environmental contaminant widely recognized as a carcinogen, mutagen and teratogen toward humans and animals. The objective was to study the toxicity induce by  $K_2Cr_2O_7$  and to evaluate the possible ameliorating effect of vitamin-C on potassium dichromate ( $K_2Cr_2O_7$ ) induced oxidative stress and generation of inflammatory response in rats. **Materials and Methods:** Adult male wistar rats were randomly divided into 4 groups of five animals each: Group I-N received single i.p., injection of normal saline and served as normal control group; group II ( $K_2Cr_2O_7$ )-K group was subjected to a single i.p., injection of potassium dichromate ( $15\text{ mg kg}^{-1}$ ) to induce toxicity; while the group III ( $K_2Cr_2O_7$ +Vitamin C)-CK group was pretreated with single i.p., injection of vitamin-C ( $250\text{ mg kg}^{-1}$ ), 6 h prior to administration of potassium dichromate. The group IV (Vitamin-C only)-C group received single i.p., injection of vitamin-C in saline ( $250\text{ mg kg}^{-1}$ ). The body weight of each animal was recorded before and after completion of the respective treatment. Oxidative stress markers like MDA, glutathione levels and serum interleukins (IL-10, IL-1 $\alpha$ , IL-6, IL-8, IL-18 and TNF- $\alpha$ ) were determined in all of rats studied. Comparison between the groups was performed by one way ANOVA followed by Holm-Sidak test. Pearson's correlation coefficient was performed to study the correlation between interleukins in K and CK group. **Results:** The  $K_2Cr_2O_7$  administration increased serum IL-6, IL-8, IL-10, IL-18 and TNF- $\alpha$  levels significantly ( $p < 0.001$ ) compared to saline treated control group. The levels of MDA and glutathione were altered significantly ( $p < 0.001$ ) in dichromate treated group compared to the control. These changes were reversed significantly ( $p < 0.001$ ) in animals receiving a pretreatment of vitamin-C. **Conclusion:** It is concluded that potassium dichromate is known to induce oxidative stress and inflammation in rats and the toxicity is reversed by the chemoprotective and anti-inflammatory property of vitamin-C.

**Key words:** Cytokines, interleukins, potassium dichromate, oxidative stress, vitamin-C, chemoprevention

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**Data Availability:** All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Oxidative stress is defined as an imbalance between pro-oxidant and antioxidant forces. Cellular toxicity due to oxygen free radical is deactivated by antioxidant defense mechanism and a balance between these two processes maintains the normal physiological state in the cell<sup>1</sup>. Reactive Oxygen Species (ROS) or free radicals especially the hydroxyl radical can cause loss of protein function, breakage of DNA, peroxidation of lipids in membrane affecting the fluidity and activity of membrane proteins. The probable detrimental effects of ROS are generally controlled by cellular endogenous antioxidant mechanisms which include glutathione and antioxidant enzymes<sup>2</sup>. Oxidative stress develops either due to production of oxygen radicals or due to enhanced lipid peroxidation exceeding the scavenging capacity of antioxidant enzymes. Oxidative stress can be studied by measuring the levels of malonaldehyde (MDA), product of lipid peroxidation and levels of antioxidants like glutathione and antioxidant enzymes<sup>3</sup>.

In addition to oxidative stress, ROS are also involved in a clinical condition called Systemic Inflammatory Response Syndrome (SIRS). Increase in oxidative stress causes increased generation of inflammatory cytokines. Cytokines are a large family of interleukins, interferons and TNF's (Tumor Necrosis Factors) and they are responsible for triggering cell apoptosis as well as inflammatory cascades. These oxygen free radicals also play a key role in onset and progression of inflammation<sup>4</sup>.

There are different mechanisms proposed for cytokine production under oxidative stress. Firstly, the transcription factors like NF $\kappa$ B and Activator Protein-1 (AP-1) are activated by oxygen derivatives and transcription of genes encoding cytokine and growth factor occurs. Secondly, in later stages combined action of free cytokines and free radicals activates endothelial cells and promotes synthesis of inflammatory mediators and adhesion molecules. Lastly, the ROS exert their toxic effect on the cell components at the site of inflammation resulting in loss of cell functioning and death. Hence, generation of cytokines is accepted to play a major role in inducing inflammatory responses in general and SIRS in particular<sup>5</sup>.

Chromium is a metal found vastly in plants, animals, soil, gases and volcanic dust. It exists as Cr (VI) or Cr (III) in aqueous solution<sup>6</sup>. These two oxidative states have different chemical, biological and environmental properties<sup>7</sup>. The Cr (III) is an essential micronutrient, on the other hand Cr (VI) is toxic in nature and a primary contaminant due to its toxicity to humans, animals, plants and microorganisms<sup>8</sup>. A hexavalent form of chromium exists in potassium dichromate (K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>)

and when linked to oxygen it forms a strong oxidizing agent with toxic and carcinogenic effects on humans and animals<sup>9</sup>. Hexavalent form of chromium mostly enters cells and it undergoes metabolic reduction to trivalent chromium, resulting in formation of reactive oxygen species; oxidative tissue damage and a cascade of cellular and immune responses<sup>10</sup>.

Of all the micronutrients, hallmark of vitamin-C is in its potential detoxifying effect on immune system and host defense mechanisms<sup>11</sup>. Supplements of vitamin-C have been shown to alter human immune responses. Vitamin-C exerts its antioxidant property by scavenging oxygen radicals and as aqueous phase peroxy<sup>12</sup>. Currently, there is limited information on the effects of vitamin-C on the production of cytokines. These all points strongly recommend study to evaluate the toxicity of potassium dichromate on the immune system and the possible chemoprotective effect of vitamin-C. Therefore, the aim of this study was to investigate oxidative stress induced by potassium dichromate and impact of vitamin-C on the synthesis of pro inflammatory cytokines (IL-1 $\alpha$ , IL-6, IL-8 and TNF- $\alpha$ ).

## MATERIALS AND METHODS

**Animals and treatments:** The present study was carried out in Animal house and Research Laboratory, College of Applied Medical Sciences, King Saud University in the month of May, 2014. Male Wister rats, weighing 170-260 g, were used in the experiment. The animals were kept under standard housing facilities (24  $\pm$  1  $^{\circ}$ C, 45  $\pm$  5% humidity and 12 h light/dark cycle). They were supplied with free access to standard laboratory chow and water and left to acclimatize for one week before the experiments. All experiments were performed in compliance with the guidelines for the care and use of laboratory animals. The animal experiments were conducted according to the guidelines of Animal ethics, King Saud University.

**Study design:** Adult male Wistar rats were randomly divided into 4 groups of five animals each: Group I-N received single i.p. injection of normal saline and served as normal control group (saline); group II (K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>)-K group was subjected to a single i.p. injection of potassium dichromate (15 mg kg<sup>-1</sup>) to induce toxicity; while the group III (K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>+Vitamin C)-CK group was pretreated with single i.p., injection of vitamin-C (250 mg kg<sup>-1</sup>), 6 h prior to administration of potassium dichromate. The group IV (Vitamin-C only)-C group received single i.p., injection of vitamin-C in saline (250 mg kg<sup>-1</sup>). The

body weight of each animal was recorded before and after completion of the respective treatment.

**Sample preparation and biochemical estimation:** Two days after potassium dichromate administration, animals were sacrificed and blood samples were collected and centrifuged for 10 min at 5000 rpm to obtain serum and stored at  $-20^{\circ}\text{C}$  for subsequent measurement of Cytokines. Serum samples were filtered prior to analysis. Serum levels of cytokines (IL-6, IL-8, IL-10, TNF- $\alpha$ , IL-1 $\alpha$ ) was estimated using MILLIPLEX MAP Cytokine/Chemokine Magnetic bead panel Kit/Multiplex assay (purchased from Millipore) and beads were read on LUMINEX<sup>200</sup>™ using Xponent software. The IL-18 and IL-1 $\beta$  was also estimated separately using ELISA kit, MBL. All measurements were conducted in duplicate. The concentrations of cytokines analyzed were expressed in  $\text{pg mL}^{-1}$ . Serum levels of malondialdehyde (MDA) and reduced GSH levels were also determined to study the oxidative stress.

**Lipid peroxidation (LPO):** The assay for lipid peroxidation was done following the method of Wright *et al.*<sup>13</sup>. The amount of malonaldehyde (MDA) formed in each sample was assessed by measuring the optical density of the supernatant at 535 nm using a spectrophotometer against a reagent blank. The results were expressed as  $\mu\text{mol L}^{-1}$ .

**Estimation of glutathione (GSH):** Reduced GSH was assayed by the method of Jallow *et al.*<sup>14</sup>. The yellow color developed was read immediately at 412 nm on a spectrophotometer. The amount of glutathione was expressed as  $\mu\text{mol L}^{-1}$ .

**Statistical analysis:** The results were expressed as Mean  $\pm$  S.D. Statistical analyses were performed using SPSS software. Comparison between the groups was performed by one way ANOVA followed by Holm-Sidak test. The correlation between mean values of IL-10, IL-1 $\alpha$ , IL-6, IL-8, IL-18 and TNF- $\alpha$  in K and CK group were evaluated with Pearson's correlation coefficient.  $p < 0.05$  was considered to be statistically significant<sup>15</sup>.

## RESULTS

**Effect of vitamin-C on potassium dichromate induced oxidative stress and inflammation:** The  $\text{K}_2\text{Cr}_2\text{O}_7$  administration to rats increased serum MDA levels

( $1.34 \pm 0.11 \mu\text{mol L}^{-1}$ ) and decreased the glutathione levels ( $0.78 \pm 0.08 \mu\text{mol L}^{-1}$ ) in serum in K group compared to control group ( $0.49 \pm 0.02$  and  $2.52 \pm 0.31 \mu\text{mol L}^{-1}$ , respectively). The pretreatment of vitamin-C to rats significantly compensated the increased MDA and reduced glutathione levels ( $p < 0.001$ ). Table 1 depicts the mean values of interleukins, TNF- $\alpha$  and oxidative stress markers in four groups (N, K, CK and vitamin-C group) and comparison between the groups is shown in Table 2.

In addition to oxidative stress markers, dichromate administration resulted in increased serum levels of cytokines like IL-10, IL-1 $\alpha$ , IL-6, IL-8, IL-18, IL-1 $\beta$  and TNF- $\alpha$  in K group of rats compared to saline treated control ( $p < 0.001$ ). Pretreatment with vitamin-C in CK group significantly decreased ( $p < 0.001$ ) the  $\text{K}_2\text{Cr}_2\text{O}_7$  induced toxicity and resulted in substantial recovery of oxidative damage by enhancing levels of antioxidants like glutathione and lowering the inflammatory cytokines in sera. Furthermore, vitamin-C treatment alone in C group, exhibited enhanced levels of antioxidants and diminished production of cytokines as compared to controls. Pretreatment with vitamin-C in CK group showed varying effects on the levels of cytokines; the levels of IL-10, IL-6, IL-8, IL-18, TNF- $\alpha$  and MDA decreased significantly ( $p < 0.001$ ) compared to K group. On contrary, the levels of IL-1 $\alpha$  and IL-1 $\beta$  were decreased but not statistically significant. Vitamin-C did not exert any prominent effect on the levels of these cytokines upon pretreatment in CK group of rats.

Further, pearsons correlation studies were performed in K and CK groups to study the inter-relationship between interleukins and TNF- $\alpha$ . Regression graphs showing correlation between the interleukins and TNF- $\alpha$  in K group are shown in Fig.1a-f. In dichromate treated group, interleukins (IL-10, IL-1 $\alpha$ , IL-6, IL-8, IL-18) and TNF- $\alpha$  were correlated with each other and were found to increase significantly ( $p < 0.05$ ) in positive association. In contrast to this, negative correlation was obtained between IL-1 $\alpha$  with IL-6, IL-8, IL-18 and TNF- $\alpha$ ; also IL-1 $\beta$  with IL-18, IL-1 $\beta$  with TNF- $\alpha$ . Regression graphs showing correlation between the interleukins and TNF- $\alpha$  in CK group are shown in Fig. 2a-f. Similarly in CK group, interleukins were positively correlated with each other. The IL-10 was positively correlated with IL-6, IL-8, IL-18 and TNF- $\alpha$  at ( $p < 0.001$ ) level of significance and with IL-1 $\alpha$  and IL-1 $\beta$  at  $p < 0.05$ . The IL-6 was positively correlated with IL-18, IL-1 $\beta$  and TNF- $\alpha$  at  $p < 0.05$  and with IL-8 at  $p < 0.001$  level of significance. IL-8 was positively correlated with IL-1 $\beta$  and was statistically not significant. The correlation between TNF- $\alpha$  and IL-1 $\beta$  was positive at  $p < 0.05$ .

Table 1: Mean values of cytokines and oxidative stress markers in four groups

Cytokines	Group I	Group II	Group III	Group IV
	Control group (N)	K (dichromate group)	Vitamin C+K	Vitamin C
IL-10	315.50±12.41	1200.00±122.6	409.01±24.0	337.48±8.710
IL-1α	1395.20±2.050	1503.60±43.32	1491.80±70.1	1425.60±28.05
IL-6	472.53±33.85	551.32±29.60	491.00±7.41	480.26±46.18
IL-8	448.40±36.60	857.58±31.05	470.64±24.52	447.36±26.38
IL-18	254.20±8.600	272.00±9.170	262.00±9.90	286.60±6.500
IL-1β	33.73±3.530	42.43±0.700	39.80±0.46	37.40±0.370
TNF-α	259.45±24.40	512.83±101.4	296.30±19.3	271.80±3700
MDA (μmol L <sup>-1</sup> )	0.49±0.020	1.34±0.110	0.96±0.01	0.52±0.010
GSH (μmol L <sup>-1</sup> )	2.52±0.310	0.78±0.080	1.50±0.25	2.20±0.150

MDA: Malonaldehyde, GSH: Glutathione, Each value represents Mean±SD

Table 2: Comparison of the cytokines between the groups by ANOVA

Cytokines	N with K		K with CK		K with C		CK with C	
	t-value	p-value	t-value	p-value	t-value	p-value	t-value	p-value
IL-10	22.21	<0.001*	19.86	<0.001*	21.66	<0.001*	1.79	0.17
IL-1α	52.27	<0.001*	0.45	0.880	52.14	<0.001*	51.74	<0.001*
IL-6	3.83	0.009**	2.93	0.030**	3.46	0.016**	0.52	0.84
IL-8	21.55	<0.001*	20.30	<0.001*	21.60	<0.001*	1.22	0.55
IL-18	4.06	0.005**	3.42	0.010**	3.04	0.030**	0.38	0.70
IL-1β	7.52	<0.001*	2.27	0.070	4.35	0.002**	2.08	0.05
TNF-α	7.11	<0.001*	6.09	<0.001*	6.78	<0.001*	0.68	0.75
MDA	22.50	<0.001*	9.90	<0.001*	21.70	<0.001*	11.80	<0.001*
GSH	12.40	<0.001*	5.16	<0.001*	10.19	<0.001*	5.02	<0.001*

t: Tabulated values, p: Probability (\*p<0.001 and \*\*p<0.05)

## DISCUSSION

The major findings of the present study are the significant increase in levels of serum IL-6, IL-8, IL-10, IL-18 and TNF-α levels on K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> administration (p<0.001) compared to saline treated control group. The levels of MDA increased and glutathione decreased significantly (p<0.001) in dichromate treated group compared to the control. These changes were reversed significantly (p<0.001) in animals receiving a pretreatment of vitamin-C. Chromium (Cr) is the seventh most abundant element on the surface of the earth<sup>16</sup>. It exists naturally in the form of chromite or in combined form with other metals forming complexes like crocoite and bentorite. Chromium is widely used in industries for plating, tanning of leather, alloying etc. Chromium is naturally present in air, water, soil and food; it can gain access in food chain through various exposure routes like ingestion, inhalation or dermal contact.

A hexavalent form of Cr is present in K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> (CrVI); the increased usage of CrVI in industries, its emission in atmosphere by catalytic converters and its improper disposal causes different health hazards. The environmental

contamination of Cr is increased as a result of its vast anthropogenic use and is has become a major area of concern<sup>17</sup>. Reports of epidemiological investigations have shown that respiratory cancers have been found in workers occupationally exposed to Cr (VI) compounds. Numerous studies carried out in humans and animals have established a strong association between industrial pollution (caused by industries manufacturing chromium compounds) and workers health. It is also suggested that Reactive Oxygen Species (ROS) are involved in Cr (VI)-induced cell injury and cancer<sup>18</sup>. Keeping in view the toxicity of Cr, in USA the Cr concentration is regulated by implementing permissible limits of Cr to 0.1 mg L<sup>-1</sup> in drinking water. The CrVI in hexavalent form is comparatively soluble; it undergoes leaching process through soil and enters groundwater. A typical ratio of 0.45% is recorded for chromium in plants to chromium in soil. The Environmental Protection Agency (EPA) has set standards for permissible limit of CrIII and CrVI to 100 μg L<sup>-1</sup> of drinking water<sup>19</sup>.

The objective of this investigation was to study the toxic effects of K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>; relationship between K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>, vitamin-C and markers of inflammation like cytokines. There is a close

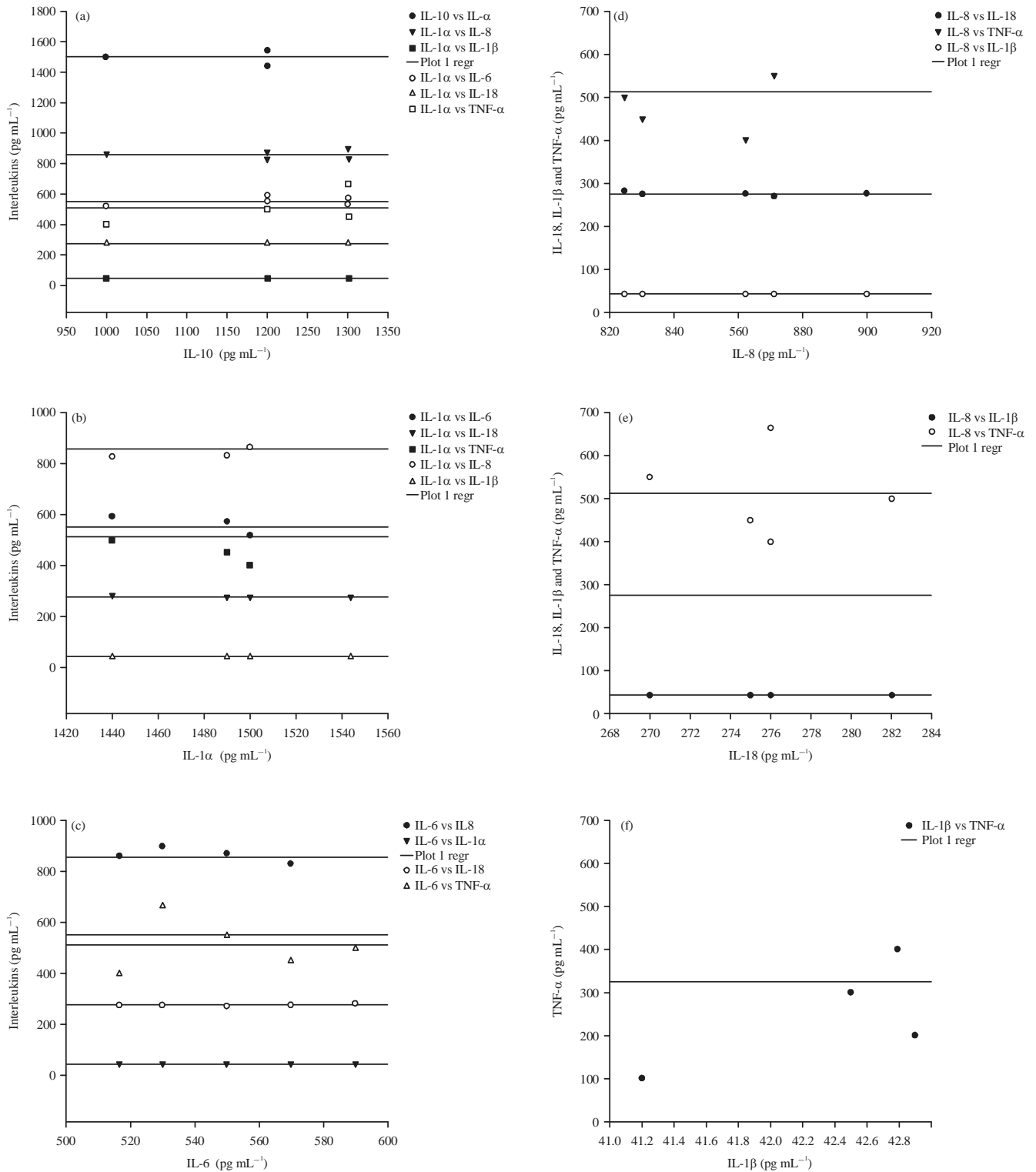


Fig. 1 (a-f): Regression graph showing correlation between vs Interleukins and TNF-α in K group (a) IL-10 and other interleukins and TNF-α in K group, (b) IL-1α with other interleukins and TNF-α in K group, (c) IL-6 with other interleukins and TNF-α in K group, (d) IL-8 with IL-18, IL-1β and TNF-α in K group, (e) IL-18 with IL-1β and TNF-α in K group and (f) TNF-α and IL-1β in K group

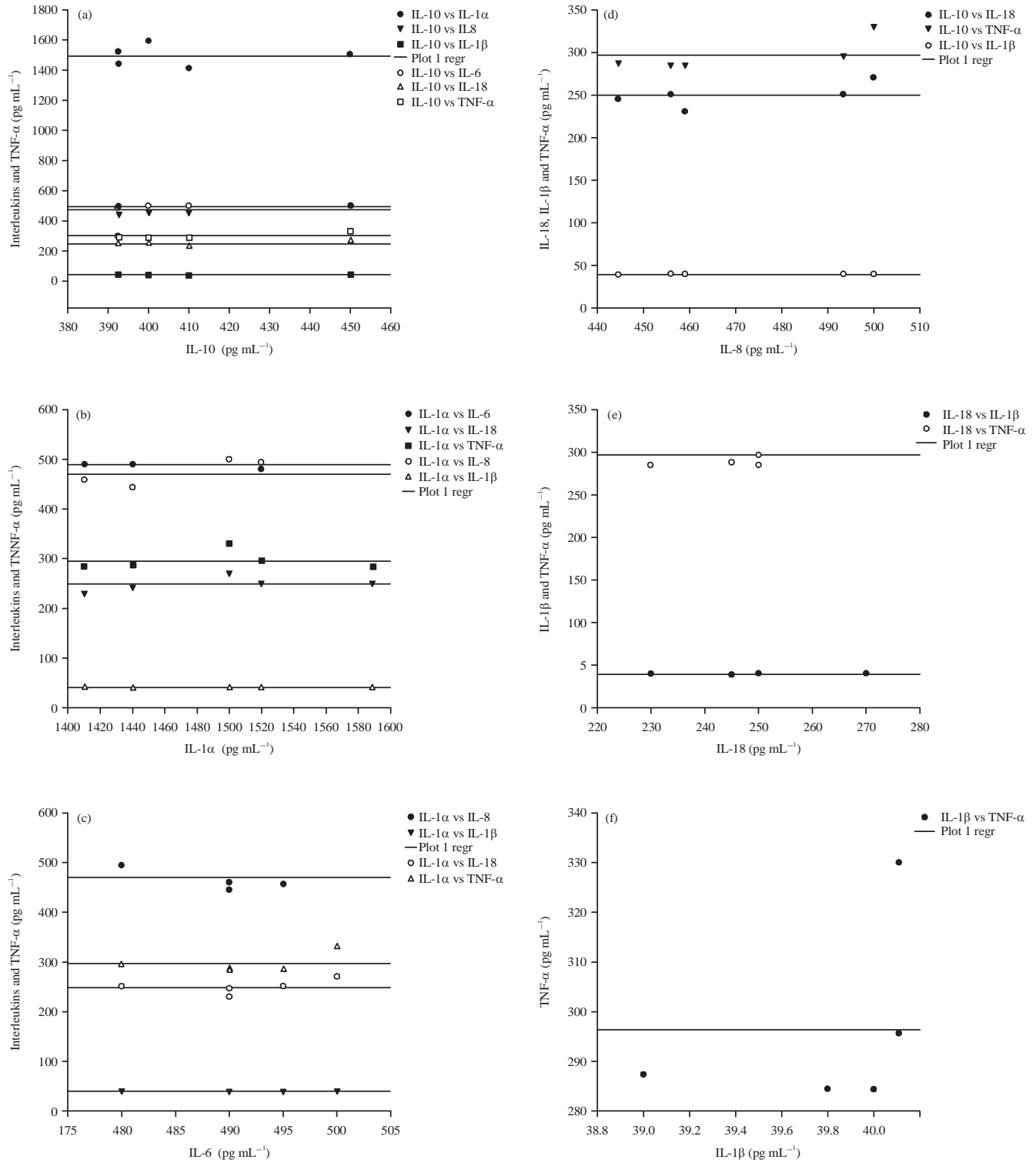


Fig. 2(a-f): Regression graphs showing correlation between Interleukins and TNF- $\alpha$  in CK group (a) IL-10 with other interleukins and TNF- $\alpha$  in CK group, (b) IL-1 $\alpha$  and other interleukins and TNF- $\alpha$  in CK group, (c) IL-6 and other interleukins and TNF- $\alpha$  in CK group, (d) IL-8 with IL-18, IL-1 $\beta$  and TNF- $\alpha$  in CK group, (e) IL-18, IL-1 $\beta$  and TNF- $\alpha$  in CK group and (f) IL-1 $\beta$  and TNF- $\alpha$  in CK group

association observed between oxidative stress and inflammation and we hypothesize that an increase in oxidative stress-derived inflammation is a major mechanism in the toxicity induced by dichromate. The present work has studied the effect of potassium dichromate on immune system, serum cytokine levels and the possible protective role of vitamin-C in rats. The process of oxidative degradation of fatty acids also called as lipid peroxidation (LPO) is a general mechanism for cellular injury and death. It can be evaluated in biological samples like serum by estimating the levels of malondialdehyde (MDA) as it is one of the stable aldehydic products of LPO<sup>13</sup>. The CrVI upon entry in cell undergoes process of rapid reduction to form ROS and Cr intermediates which directly interact with cellular constituents<sup>20</sup>. The other forms of Cr (CrV, CrVI and CrIII) also have the ability to generate free radicals like peroxy nitrite, NO, hydroxyl and superoxide leading to damage similar to oxidative stress<sup>21</sup>. Free radicals can enhance MDA levels which is a major product of LPO, by effectively damaging almost all types of macromolecules including lipids. In a study by Soudani *et al.*<sup>22</sup>, K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> treatment increased the amount of MDA levels in serum, suggesting oxidative stress. Similar to these results, in this study increased MDA levels in group of rats administered with K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> was observed. In contrast, under normal physiological condition, cells harbour glutathione an antioxidant present in millimolar concentrations which protects the cellular functioning against the deleterious effects of lipid peroxidation and also helps in maintaining the redox status. Diminution in levels of glutathione alarms the onset of oxidative stress. Hence, Glutathione is used a marker of oxidative stress. The observed decreased levels of antioxidant glutathione due to dichromate toxicity in rats are also in line with that observed by Rasool *et al.*<sup>23</sup>. The decrease in levels of GSH and increased lipid peroxidation are indicators of oxidative stress that resulted due to dichromate toxicity.

In addition to enhanced lipid peroxidation and decreased antioxidant status, the toxicity of potassium dichromate can also be explained by inflammation as a result of enhanced content of TNF- $\alpha$  and pro-inflammatory cytokines. Hexavalent chromium can trigger multiple pathways- it increases ROS formation; activates NF- $\kappa$ B, Akt and MAPK pathways; increases cytokines production particularly TNF- $\alpha$  and IL-1 $\alpha$ <sup>24</sup>. Similar to this study, toxic nature of potassium dichromate and its ability to induce oxidative stress is also reported by other workers<sup>25</sup>. Cytokines play a crucial role by activating cell apoptosis, inflammatory cascades and as mediators in SIRS progression<sup>26</sup>. In particular TNF- $\alpha$ , IL-1 $\beta$ , IL-6 and IL-8 appear early in circulating blood in a classical sequence. The IL-18 is known to initiate cascade of events involving cellular targets like macrophages, T cells and NK cells, which leads to

inflammation, triggers apoptosis of endothelial cells and induces enhanced expression of ICAM-1 and subsequent cellular injury<sup>27</sup>. The IL-18 was earlier identified as a factor which together with T helper 1 cells (Th1) and IL-12 increases production of IFN- $\gamma$ . However its activity alone or in combination with IL-2 triggers T helper 2 cell response, which is also called as Th2 response. The Th2 effect of IL-18 includes production of IL-6 by CD4+T cells, basophils and mast cells<sup>28</sup>. In the present study, the elevated level of IL-18 in dichromate-treated group is accompanied by elevated level of IL-6, IL-8, IL-10. These results suggest that IL-18 acted through stimulation of Th2 response and production of other interleukins. Production of interleukins was found to be accompanied with increased production of TNF- $\alpha$ . In the present study the observed positive correlation between IL-18 and IL-6 may account for raised levels of these interleukins. It was also observed that TNF- $\alpha$  was positively correlated with interleukins. This positive correlation between them may account for raised levels of cytokines in serum of rats.

The TNF- $\alpha$  is mainly produced by monocytes and macrophages, it is a cytokine with pleiotropic effects- it is involved in systemic inflammation; it triggers a cascade of cytokines, increases vascular permeability, divert macrophages and neutrophils to the site of infection resulting in local inflammation<sup>29</sup>. The TNF- $\alpha$  triggers NF- $\kappa$ B signaling which regulates transcription of genes producing different cytokines which are involved in cell adhesion, cell proliferation, cell survival, inflammatory response and anti-apoptotic factors<sup>30,31</sup>. Similar results were obtained in this study where a positive correlation was observed between TNF- $\alpha$  and cytokines. The relationship between oxidative stress and TNF- $\alpha$  is complex and it has been shown that TNF- $\alpha$  increases ROS and ROS increases TNF- $\alpha$  level<sup>32</sup>. Young *et al.*<sup>33</sup> proposed that H<sub>2</sub>O<sub>2</sub> can induce expression of TNF- $\alpha$  in human keratinocytes. It has been also shown that TNF- $\alpha$  through activation of NF- $\kappa$ B enhances ROS production<sup>34</sup>. These results indicate a possible positive association between NF- $\kappa$ B activation, formation of ROS and expression of TNF- $\alpha$ . Another positive reciprocal association has been demonstrated between TNF- $\alpha$  and IL-1. It was observed that expression of TNF- $\alpha$  leads to secretion of IL-1 via activation of TAK1, NF- $\kappa$ B and MAPK pathways. In addition IL-1 increases expression of TNF- $\alpha$  via activation of TAK1, NF- $\kappa$ B and MAPK pathways<sup>35-37</sup>. Furthermore, in certain cell types it was observed that IL-1 increases ROS formation<sup>38</sup>. These all reports suggest a positive association between the release of IL-1, TNF- $\alpha$  and ROS formation.

The IL-6 is a cytokine with multiple roles, (a) it induces synthesis of acute phase proteins like CRP, fibrinogen, serum amyloid A and hepcidin in hepatocytes, (b) it inhibits albumin



production and (c) it stimulates antibody production and development of effector T-cells thereby playing an important role in acquired immune response. Additionally, IL-6 can boost up proliferation of non-immune cells. As a result of these multiple roles, dysregulated continuous production of IL-6 leads to onset of various diseases<sup>39</sup>. Several immune cells can produce IL-10 like macrophages, B cells, T cells, mast cells, keratinocytes and few tumor cell lines. The IL-10 exerts inhibitory effect on T cells affecting functioning of macrophages and monocytes. The IL-10 was earlier known as cytokine synthesis inhibiting factor and is mostly produced by Th2 cells and CD8+ cell clones. It is capable of inhibiting synthesis of several cytokines from different cells<sup>40</sup>. Here, we reported that the IL-10 may sometime acts as proinflammatory cytokine promoting inflammation under oxidative stress and enhance the production of other inflammatory cytokines. The IL-1 $\alpha$  and IL-1 $\beta$  are equally strong inflammatory cytokines which activates inflammation and their deregulated signaling leads to severe diseases manifested by severe acute or chronic inflammation<sup>41</sup>.

Vitamin-C with its diverse effects on immune system is known to be an important physiological antioxidant<sup>42</sup>. The results obtained in current study also clearly indicate the antioxidant potential of vitamin-C against K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> induced toxicity; due to observed decrease in lipid peroxidation and increased GSH levels in vitamin-C pretreated group of rats. It has been shown by some of the earlier researchers that vitamin-C can activate immune system in response to toxins by enhancing proliferation of T-lymphocytes leading to increased production of immunoglobulins and cytokines<sup>43</sup>. Another potential mechanism reported for vitamin-C mediated enhanced immune response is inhibition of T cell apoptosis signaling pathway<sup>44</sup>. In the present study, vitamin-C selectively influences cytokine production in rats. Pretreatment to rats with vitamin-C decreased the interleukins levels of IL-10, IL-6, IL-8, IL-18 and TNF- $\alpha$ . However vitamin-C does not revert the level of IL-1 $\alpha$  and 1 $\beta$  to normal indicating that the interleukins (IL-10, IL-6, IL-8, IL-18) and TNF- $\alpha$  are possible markers of inflammation induced by dichromate toxicity. Vitamin-C may inhibits the activation of transcription factor NF- $\kappa$ B, which plays a critical role in the production of inflammatory cytokines such as TNF- $\alpha$ , IL-1, IL-6, IL-8, IL-18, IL-10. This study suggests that vitamin-C may play a significant role in the regulation of the inflammatory response.

## CONCLUSION

The results obtained in this study conclude that oxidative stress and inflammation play a key role in toxicity induced by

potassium dichromate and hazardous effects of dichromate are ameliorated in presence of vitamin-C that suppresses inflammation. The major role of vitamin C is found in its effectiveness to reduce serum cytokines and protect cellular membrane damage against the deleterious effect of ROS. In summary, present data suggests that potassium dichromate is a potent chemical contaminant in atmosphere and its toxicity may be reduced by vitamin-C which is an effective chemo preventive and anti-inflammatory agent.

## SIGNIFICANCE STATEMENTS

This study discovers the chemopreventive and anti-inflammatory properties of vitamin C that can be beneficial for diminishing the toxicity induced by dichromate. This study will help the researchers to uncover the critical areas of inflammation and oxidative stress in which vitamin C plays a major role as antioxidant and anti-inflammatory agent, that many researchers were not able to explore. Thus a new theory on the role of vitamin C and inflammatory cytokines, may be arrived at.

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