Protective effects of vitamin C on albino rats Exposed to hexavalent chromium (Cr VI)

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Abstract:
Hexavalent chromium compounds are widely recognized as carcinogenic agent. Chromium intoxication is probably one of the most common forms of metals intoxication. Vitamin C has been reported as one of the more potent reductant of CrVI. The Exposure of rats to CrVI as (potassium dichromate) for 2 months day by day (25ppm (CrVI) in dinking water), induced an alterations in some biochemical parameters. Vit. C administration (150mg/l) in drinking water in combination with CrVI had prevented most of alterations induced by CrVI.

Elevation of aspartate aminotransferase (SAT), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) activities in the blood and liver tissue, were recorded in the rats exposed to CrVI. Although most of these activities decreased near to control due to used Vit C. It's suggested that Vit.C. Administration could prevent toxic effects of Cr.VI probably by its anti-oxidant properties.

Introduction:
Hexavalent chromium compounds are widely recognized as human carcinogens (De flora et al., 1990) It has been suggested to cause the generation of reactive oxygen species (ROS) resulting in oxidative stress, alter the antioxidant defense system in the tissues and cellular injury, which may be one of the factors in the etiology of cancer (Sha et al., 1999 and Bagchi, 2001)
Chromium (vi) compounds have been reported to be more toxic and carcinogenic than chromium (iii) compounds (Bianchi et al., 1983 and De Flora et al., 1989), because the former can pass through cell membranes more easily than the latter. However, once inside cells, chromium (vi) is believed to be subsequently reduced through intermediates to chromium (iii) by cellular reductant including glutathione, cysteine, hydrogen peroxide and vitamins, such as ascorbic acid. (Cupo et al., 1982; Kitagawa et al., 1988; and Comet & Wetterhahn, 1983)

Ascorbic acid (vit. C) is an important biological reductant in humans and animals and has been reported to be present normally in millimolar concentration in humans and animals tissues (Horning, 1975), and to be a more potent reductant of Cr (vi) than glutathione under physiological condition (Suzuki & Fukuda, 1990). Recent work has shown that ascorbate (vit. C) is the principle reductant of Cr (vi) in rat kidney, liver and lung ultrafiltrateS (Standeven and Wetterhahn, 1991, 1992). It is not yet fully understood whether the intracellular reduction of Cr (vi) by ascorbate is toxification or detoxification process (Diane et al., 1994).

In the present study, we examined the possible protective effect of vit. C on albino rats exposed to Cr (vi) through study some biochemical indices.

Materials & Methods:
Thirty male rats weighing 220-270 gm in average were housed in standard cages, where feed and water were supplied ad libitum. After two weeks of acclimation, animals were divided into three groups as follows:
First group: served as control and received distilled water as vehicle.
Second group: was administered 20 ppm Cr (vi) (as chromium dichromate) in drinking water for 2 months.
Third group: was received 20 ppm Cr (vi) plus vitamin C (150 mg\L) in drinking water for 2 months.
Enzyme assessments:
At the end of the experimental period, rat were fasted for 12 hours, and then sacrificed by cervical decapitation and fasting blood samples were collected from the sacrificed animals in tubes with heparin. Plasma samples were obtained by centrifugation at 860 xg for 20 minutes and stored at -20 C till measurements. Also, liver was immediately removed, weighed and washed using chilled saline solution. Liver was minced and homogenized (10% w/v), separately, in ice-cold 1.15% KCl-0.01 M sodium, potassium phosphate buffer (PH 7.4) in a Potter-Elvehjem type homogenizer. The homogenate was centrifuged at 10.000 xg for 20 minutes at 4 C and the resultant supernatant was used for different enzyme assays.
Plasma and liver Aspartate aminotransferase (AST; EC 2.6.1.1) and Alanine aminotransferase (ALT; EC 2.6.1.2) activities were determined with kits from (Randux). The principle reaction of the colorimetric determination of AST or ALT activity is based on the reaction of Aspartate or Alanine with alpha-ketoglutarate to form oxaloacetate or pyruvate, respectively. The oxaloacetate or pyruvate formed is measured by monitoring the concentration of oxalacetate or pyruvate hydrazone formed with 2,4-dimtrophenylhydrazine.
Alkaline phosphatase (ALP; EC 3.1.3.1) activity was measured at 405 nm by the formation of paranitrophenol from paranitrophenylphosphate as a substrate (Principato et al., 1985).

Data analysis: Data were computer analyzed using SPSS ver 14.0 for windows (statistical package for the social sciences In, Chicago, Illinois). Differences among groups were assessed by one way ANOVA.

Results:
The effect of chromium and chromium plus ascorbic acid on serum AST, ALT and ALP level are present in table (1).

Activities of serum AST, ALT and ALP were increased significantly in the rat received Cr (vi), on the other hand these activities in the rats received a combination of Cr (vi) and ascorbic acid remained near the control value.

Table (1): Assay of plasma enzymes activities.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Cr (vi)</th>
<th>Cr (vi) + vit. C</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (udL⁻¹)</td>
<td>34.2±0.5</td>
<td>58.0±2.3*</td>
<td>36.4±0.1</td>
</tr>
<tr>
<td>ALT (udL⁻¹)</td>
<td>68.7±0.7</td>
<td>92.0±0.8*</td>
<td>36.4±0.1</td>
</tr>
<tr>
<td>ALP (uL⁻¹)</td>
<td>187.1±4.6</td>
<td>212.0±5.2*</td>
<td>169.1±2.1</td>
</tr>
</tbody>
</table>

* Significant differences at P<0.05

In addition, the activities of AST, ALT and ALP were also significantly (P<0.05) increased in the liver tissues of Cr (vi) exposed rat compared to the control value (table 2).

Table (2): Assay of liver tissue enzymes activities.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Cr (vi)</th>
<th>Cr (vi) + vit. C</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (udL⁻¹)</td>
<td>161±4.1</td>
<td>194±6*</td>
<td>172±4</td>
</tr>
<tr>
<td>ALT (udL⁻¹)</td>
<td>210±5.9</td>
<td>263±8*</td>
<td>209±9</td>
</tr>
<tr>
<td>ALP (uL⁻¹)</td>
<td>224±12</td>
<td>292±11*</td>
<td>231±14</td>
</tr>
</tbody>
</table>

* Significant differences at P<0.05

Discussion:
Cr (vi) fed to albino rats, in drinking water, for 2 months produced harmful changes in the studied biochemical blood and liver tissue parameters. However, these changes showed signs of improvements with the treatments with vitamin C compared to Cr (vi) treated rats alone.

Serum transaminases (AST & ALT) and alkaline phosphatase exhibited a general increase in the blood and liver tissue of Cr (vi) treated rats compared to control. The observed elevation in enzymes activities in response to Cr (vi) administration is in agreement with previous studied of (Kim & Ma, 1991 and Mosaad et al., 2004). The liver enzymes are normally found in circulation in small amounts because of hepatic growth and repair. As a liver specific enzymes ALT only significantly elevated in hepatobiliary disease.
Increase in AST levels, however, can occur in connection with damages of heart or skeletal muscles as well as of liver parenchyma. (Moss & Henderson, 1999)

Consequently, elevated activities of ALT and AST observed in the current study in response to Cr (vi) administration could be a common sign of impaired liver function.

It is evident from the results of the present investigation that administration of vit. C at a dose of 150 mg/L can lead to a more pronounced decrease in the activity of ALT and AST in the serum and liver tissue provide significant restoration of vit. C. reduced oxidative stress.

Little et al. (1996) administered several drugs to screen for their potential to protect keratinocytes against the cytotoxic effect of Cr (vi), they concluded that only ascorbic acid offered complete protection.

On the other hand, Sally and Allister, 1999, based on experimental studies, involving administration of ascorbic acid in the treatment of systemic chromium poisoning and chromium dermatitis, concluded that, there is in sufficient evidence to advocate the use of ascorbic acid may reduce dermal hexavalent chromium exposure, but this observation must be confirmed in controlled studies.

Suzuki (1988), used vit. C for reduction of Cr (vi) in rat lung lavage fluid, suggested that the living layers (surfactant layers) of rat lungs provide an ascorbic acid related capacity for protection of the cells against the toxic effects of chromates and probably other oxidants.

In conclusion, our findings suggested that the presence of vit. C with Cr (vi) restored the changes in enzyme activities and according to that, vit. C could be effective in the protection of chromium-induced toxicity.

References: