Histopathological Changes on Liver Architecture Due to Toxic Effect of Chromium with Protective Role of Vitamin E on Albino Rats: A Prospective Study

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Abstract

Introduction This histopathological study was done with an aim to learn about the biohazardous effects of chromium toxicity with protective role of alpha-tocopherol on liver parenchyma of albino rat when fed orally.

Materials and Methods The study was conducted in the Department of Anatomy at Subharti Medical College, Meerut (Uttar Pradesh), India, in collaboration with Department of Pathology and Pharmacology.

The experiment was conducted as per the guidelines of the Committee for Purpose of Control and Supervision of Experiments on Animals, with registration number 1204/PO/Re/S/2008/CPSEA, and with permission of the Animal Ethical Committee, on 36 adult male rats of ~60 days of age; 140 ± 20 g of body weight was used; all the experimental animals were kept in polypropylene cage, and fed with pellet diet and water.

Result When exposed to chromium for long, it resulted in toxicity and damaged the cellular components during reduction process of chromium by releasing of free radicals, leading to deoxyribonucleic acid damage, but the chain-breaking antioxidant effects of alpha-tocopherol played an important role by reversing the damage caused by chromium toxicity, hence proved hepatoprotective.

Conclusion Vitamin E (alpha-tocopherol) is proved to be hepatoprotective against hepatotoxic effect of chromium metal.

Keywords
► chromium
► vitamin E
► Kupffer cells
► periportal necrosis
► Z-proportion test

Introduction

Liver is an organ for metabolism, detoxification, and secretory functions. It is open to hepatotoxicity through an oxidative stress mediated mechanism1 where there is increase in hepato- malondialdehyde (MDA) and decrease in protective glutathione reductase (GSH) levels.2

Heavy metals like chromium when exposed to an organism (mammals) for long term result in slow progressive physical, muscular, neurological, and degenerative changes,3 along with the changes in blood composition as it is toxic and carcinogenic in nature. Chromium (Cr), a heavy metal with atomic number 24, is a hard, brittle, corrosive element with...
high melting point,\textsuperscript{4} and occurs in two valence states in nature: trivalent chromium, Cr(III), and hexavalent chromium, Cr (VI). 

Cr(VI) compounds have been reported to be more toxic and more carcinogenic than Cr(III), because once Cr(VI) passes through the cell membrane, it reduces to its lower oxidation state and generates reactive oxygen species (ROS).\textsuperscript{5} ROS causes injury to proteins, lipids, and deoxyribonucleic acid, leading to cellular oxidative stress,\textsuperscript{6} which causes oxidative stress mechanism and damages the organ. Alpha-tocopherol (vitamin E), an antioxidant, has been reported by many studies to be protective against hepatotoxicity by reducing the levels of MDA and increasing the levels of GSH and hence reversing the damage caused by chromium toxicity.

Materials and Methods

Study Design

The analytical study was conducted on 36 adult albino rats of \~60 days of age, having 140 ± 20 g of body weight, in the Department of Anatomy at Subharti Medical College, Meerut (Uttar Pradesh), India, with approval, and following the study protocol, of the Committee for Purpose of Control and Supervision of Experiments on Animals, registration number 1204/PO/Re/S/2008/CPSEA, and with permission of the Institutional Animal Ethics Committee (IAEC). The study was conducted to compare the hepatotoxic effects of chromium metal against hepatoprotective effects of alpha-tocopherol.

Study Setting

Cr is extracted from potassium dichromate (K2Cr2O7), which dissolves in distilled water to get stock solution of 10 mg/kg body weight. This is further diluted in normal saline to get the required dose.

Alpha-tocopherol (vitamin E) is used in concentration of 125 mg/kg body weight.

For the study, 36 male albino rats of \~60 days of age and 140 ± 20 g of body weight were used.

All the experimental animals were kept in polypropylene cage, and fed with pellet diet and water.

Method

Four sections were made among the experimental animals, comprising of nine experimental animals in each section.

1. Section I: Nine animals as Control Group, kept on pellet and water.
2. Section II: Nine experimental animals for studying chromium toxicity; fed on stock solution of potassium dichromate with measure of 10 mg/kg body weight.
3. Section III: Nine animals for studying the combined effect of chromium with alpha-tocopherol (vitamin E).
4. Section IV: Nine animals to study the effect of alphatocopherol (protective effect) given as 125 mg/kg body weight.

Sections II and III were further divided into subsections as a, b, and c:

- a: drug is given for 1 day.
- b: drug is given for 2 weeks (14 days).
- c: drug is given for 6 weeks (42 days).

Section I: Only distilled water is given.

Section II:

- IIa: Acute; 10 mg/kg of potassium dichromate given orally as single dose.
- IIb: Subacute; 10 mg/kg of potassium dichromate dissolved in distilled water given for 2 weeks.
- IIc: Chronic group; where 10 mg/kg of potassium dichromate in distilled water given for 6 weeks.

Section III: Here, 10 mg/kg body weight of potassium dichromate along with 125 mg/kg body weight of alphatocopherol given orally.

- IIIa: Acute; drugs given for 1 day.
- IIIb: Chronic; drugs given for 6 weeks.

Section IV: Here, 125 mg/kg of alpha-tocopherol was given to the experimental animals for a period of 2 weeks by oral route.

Sacrificing of experimental animals was done within 24 hours of the last dose consumed, by stoking the dorsal aspect of the head. Animals were then dissected; livers were taken out and preserved in 10% buffered formalin. Sections were then prepared, stained with H&E and stained, and mounted and observed for the histopathological changes such as lymphatic infiltration, vascular congestion, cloudy swelling, and periportal necrosis.

Sample Size

With permission of the IAEC, with registration number 1204/PO/Re/S/2008/CPSEA, 36 animals were selected for the study.

Statistical Analysis

After collecting the result and analysis performed throughout the research, the statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) software, version 21, over 30 nonoverlapping fields per stained slides, and Z-proportion test was used for statistical significance, which was \( p<0.05 \) (Table 1).

Result

Thirty nonoverlapping fields per stained slides were examined; SPSS 21 software version was used for statistical analysis and Z-proportion test was used for statistical significance, which was \( p<0.05 \) (Table 1).

Section I (\textsuperscript{\textbullet} Fig. 1): This is the control group that shows normal architecture of liver parenchyma. The lobes are separated by connective tissue septa, with portal triad and the central vein lined by endothelium.

Sinusoids separate hepatic plates; hepatocytes appear polyhedral with central rounded nucleus surrounded by basophilic granules.
**Section II (~Fig. 2):** This is the chromium toxicity group where potassium dichromate is given orally and has been assorted in subsections as follows:

- **a:** Acute; the subsection that received potassium dichromate for 1 day.
- **b:** Subacute; the subsection that received potassium dichromate for 14 days.
- **c:** Chronic; the subsection that received potassium dichromate for 42 days.

**Subsections:**

- Acute (a): with normal histological liver parenchyma; hepatic lobules are separated by connective tissue septa; portal triad and central vein are lined by endothelium; sinusoids separate the hepatic plates.
- Subacute (b): shows lymphatic infiltration and fatty changes with marginal cloudy swelling in liver parenchyma.
- Chronic (c): here there is presence of congested liver parenchyma with distorted endothelial lining of blood vessels, dense lymphatic infiltration, and presence of numerous vacuoles showing fatty changes with eccentric nucleus. Kupffer cells proliferation and swelling of the cells are found due to cellular hypoxia, which damages the membrane pump leading to cloudy swelling. Also, distorted liver parenchyma due to inflammation extending up to the periportal region is seen causing dissolution of cell content leading to periportal necrosis.

**Section III (~Fig. 3):** This section shows chromium toxicity with protective effect of vitamin E; here liver parenchyma has almost reduced and reverses to almost histopathological architecture.

**Section IV (~Fig. 4):** This section shows protective effect of vitamin E; it shows normal histological pattern of liver parenchyma with hepatic plates separated by sinusoids; the portal triad and central veins are with normal endothelial lining. Lobules are separated by connective tissue septa; hepatocytes appear polyhedral with centrally placed nucleus surrounded by basophilic granules.

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**Table 1** Comparison by Z-proportion test of different histological components among the two sections

<table>
<thead>
<tr>
<th>Findings</th>
<th>Section II: Cr toxicity (%)</th>
<th>Section III: Cr + vitamin E (%)</th>
<th>Value difference</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphatic infiltration</td>
<td>62.2</td>
<td>37.4</td>
<td>24.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Vascular congestion</td>
<td>68.9</td>
<td>31.1</td>
<td>37.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Kupffer cell proliferation</td>
<td>37.8</td>
<td>21.9</td>
<td>15.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cloudy swelling</td>
<td>61.1</td>
<td>11.1</td>
<td>50.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Periportal necrosis</td>
<td>20.4</td>
<td>2.2</td>
<td>18.2</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Note: p-Value < 0.001: significant.

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**Fig. 1** The control group with normal architecture of liver parenchyma under ×400 (hematoxylin and eosin stain).
Discussion

Chromium, an essential trace element, is needed for various physiological carbohydrate metabolism in human.

The trace elements are needed for various physiological and biochemical actions such as transport across membrane, conduction of nerves, contraction of muscles, and also in synthesis and stabilization of the nucleic acid and proteins.

Exposure to entry of chromium is via ingestion, inhalation, and skin absorption.

Depending upon the valence state, solubility in water, and transient time, the hexavalent chromium uptake is more
than the trivalent chromium and is taken up by erythrocytes more readily than trivalent chromium.

Dietary intake of chromium is between 40 and 220 µg/24 hours. Chromium intake increases using stainless steel ware.

Overdose of chromium(VI), being highly toxic, is said to be carcinogenic. The toxicity of chromium(VI) plays an important role in penetrating all the body tissues and organs; hence, the palliative role of vitamin E on its toxicity.

Previous studies of Acharya et al., Da Silva et al., and Patolla and Barnes found distortion and damage of Kupffer cells, and cloudy swelling leading to necrotic changes.

Soudani et al. have shown that supplementation of antioxidant leads to reversal of the damaged hepatocytes to normal. Vitamin E, an antioxidant that eliminates lipid peroxyl and alkoxyl radicals, breaks the chain reaction of peroxidation of lipids, thus reversing altered histology.

Hence it is proved that vitamin E is hepatoprotective against metallic toxicity as an antioxidant enzyme. The study was similar to the study of Dwivedi and Jena.

In the study done, the toxic effect of chromium along with protective effect of vitamin E, when given together with chromium, was seen. Also, the damaging and distorting effect of chromium toxicity was reversed almost to the normal state, which related to the hepatocytic architecture.

Limitation of Study
None.

Human Subject
Not involved.

Animal Subject
Thirty-six albino rats were involved and sacrificed for the study with the permission of IAEC.

Conclusion
Toxic effect of chromium metal used in various industries such as steel, paints, or leather tanning is proven to be a health hazard. Its exposure leads to various neurological, muscular, gastric, dermal, and hepatic deficiencies.

The chain-breaking antioxidant vitamin E plays an important role in reversing the damages caused by the chromium toxicity, hence proved hepatoprotective.

In the study done, protective effect of vitamin E, which feeds on free radicals, reverses the damages caused by the chromium(VI) toxicity, hence proved hepatoprotective.

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None.

Conflict of Interest
None.

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Fig. 4 Protective effect of alpha-tocopherol with normal histological pattern of liver parenchyma under ×400 (hematoxylin and eosin stain).
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