

# Histopathological effects of Chromium (III) Sulfate on Liver and Kidney of Swiss Albino Mice (*Mus musculus*)

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*Abstract – Chromium (III) sulfate has various industrial applications and is widely used in leather industry due to its high tanning properties. Cr (III) is required for efficient metabolism of fats and carbohydrates in traces. Various studies have reported that its constant exposure may lead to severe health problems in both animals and humans. In this study, histopathological effect of dietary Cr (III) was evaluated on liver and kidneys of rodents. For this purpose, adult Swiss albino mice (n=25) were divided into different treatment and control groups (n=5/group) after sufficient acclimatization. After 3 weeks of treatment, animals were sacrificed and observations regarding histopathology of liver and kidneys were made in all treatment groups and compared to control. Microscopy and photography was performed after processing the tissues according to standard protocol of sectioning and staining. Liver cross sections of treated animals showed signs of fibrosis in portal area, and congestion of sinusoid and central vein. Whereas, more pronounced effects of Cr (III) toxicity were observed in kidneys. These include mononuclear cell infiltration, necrosis and contraction of glomerulus within Bowman's capsule. However, No pathological changes were observed in control group. These results support the hypothesis that enhanced level of Cr (III) contamination of food can induce both hepatotoxicity and nephrotoxicity. These basic findings prove that currently increasing levels of trivalent chromium in environment are hazardous to living organisms. Therefore, to avoid health risks to both animals and humans, conversion of toxic chromium waste to less toxic compounds is required. Moreover, exposure level through any route should also be minimized.*

**Keywords** – Chromium toxicity, Histopathology, sinusoid and central vein congestion, Swiss Albino mice, mononuclear infiltration

## INTRODUCTION

Chromium (Cr) is naturally found only in complex forms which account for 0.1-0.3 mg/kg of the Earth's crust. It occurs in several oxidation states i.e. Cr (-II) to Cr (+VI) [1]. Trivalent chromium is the most abundant form found in natural environment while hexavalent chromium is released from industries. Chromium is generally found in the form of halides, oxides or sulphides [2]. In living organisms, chromium exists in the stable trivalent state. Cr (III) can be distinguished from Cr (VI) on the basis of its low reactivity and less permeability to penetrate plasma membranes [3]. It is an essential element for plants and animals, when consumed in lower concentrations but it has harmful effects in higher concentrations [4].

Biological effects of chromium compounds can be determined through their oxidation state and solubility [5]. Such as Cr (III) is used in nutritional supplements and is considered as an essential trace element for both humans and animals [6]. While, hexavalent chromium Cr (VI), is highly toxic state which is released in emissions of several industrial processes including electroplating, leather tanning and pigment manufacture [7]. Investigations have revealed that Cr (VI) compounds induce greater toxic effects than Cr (III) compounds in rabbits.

The accumulation of metal is also higher in animals which are exposed to chromium (VI) than in those exposed to Cr (III). But both forms of chromium induce significant morphological changes in liver and alter the levels of various chemical constituents of

blood and serum [8]. Present study was designed to analyze the histopathological effects of chromium (III) sulfate on liver and kidneys of Swiss Albino mice.

### OBJECTIVES OF THE STUDY

The current study was carried out to evaluate the toxic potential of chromium sulphate absorbed through digestive tract in rodents. Liver and kidneys of albino mice were analysed for morphological abnormalities. Furthermore, histological features were analysed through microscopy of slides prepared through standard techniques of Microtomy and staining.

### MATERIALS AND METHODS

#### Ethical approval

Ethical approval for present study was obtained from "Ethics Committee" of University of Gujrat on use of Animals for scientific research.

#### Procurement and maintenance of Animals

Healthy adult mice of either sex (Swiss Albino; average body weight =  $33.52 \pm 5.49$ ; n = 25) were procured from Laboratory of Zoology Department, University of Gujrat. These were housed in appropriate cages and provided with standard rodent chow and water *ad libitum*. Five mice were housed per cage and maintained at 12h:12h light/dark photoperiod. Temperature was maintained at  $25 \pm 2^\circ\text{C}$ .

#### Heavy metal used

Heavy metal used was chromium which was in the form of chromium sulfate. Mice were fed with diet supplemented with different doses of chromium sulfate.

Formula :  $\text{Cr}_2(\text{SO}_4)_3$   
 Mol. Wt. : 392.1820 g/mol  
 Manufacturer : E. Merck Company, D-6100 Darmstadt, F.R. Germany.

#### Experimental Design

Experiment was carried out for 3 weeks from April 23, 2012 to May 13, 2012. Mice were divided into different control and treatment groups. Treatment groups were provided with diet supplemented with different doses of chromium sulfate as 2.5, 3, 3.5 and 4 gm/kg of feed for consecutive 21 days. Whereas, control group received chromium free feed.

#### Sampling

Animals were humanely sacrificed on 22<sup>nd</sup> day of experiment for collection of liver and kidney. Gross pathological examination of liver and kidney from mice of each group was conducted by considering the parameters including Size (Normal or Hypertrophic), Colour (Normal or discoloration), any growth and Cirrhosis.

#### Rinsing and slicing of organs

Organs of the mice eviscerated were rinsed with 0.085% saline soln. to remove any dirt or blood. Organs were cut into 3-4 mm thick slices.

#### Fixation of tissues

Fixation is the first step in which tissue is preserved for histological study. In the present study, Bouin's fluid was used as a fixative. Tissues were kept in Bouin's fluid for 8 hours. Bouin's fluid killed the tissue, as well as any bacteria that can cause the tissue to rot. It also coagulated and cross-linked proteins, making them insoluble; hence proteins and cellular structures were preserved.

#### Processing of tissues

Tissue samples were processed for histopathological examination as follows:

#### Dehydration

Tissues were dehydrated through a series of alcohols. The following schedule was used.

70% alcohol	1-2 hours
95% alcohol	1-2 hours
100% alcohol (I)	½-1 hour
100% alcohol (II)	½-1 hour

#### Clearing

After the graded series of alcohol tissues were cleared, pure xylene was used twice as a clearing agent, which is miscible with both 100% alcohol and paraffin wax. Tissues were kept in xylene in the following manner.

Pure xylene	3 hours
Pure xylene	3 hours

#### Wax impregnation

Paraffin wax was melted and maintained at a temperature of  $56-58^\circ\text{C}$ . Tissues were transferred to vials containing molten paraffin wax.

Paraffin wax $56-58^\circ\text{C}$	6 hours
Paraffin wax $56-58^\circ\text{C}$	6 hours

### **Embedding**

Wax molds were filled with paraffin wax and the tissues were placed in the mold. Tissues were positioned in the center of mold in proper orientation. When paraffin wax solidified, molds were transferred to a bowl of cool water. Hard blocks were removed from water and stored in the refrigerator overnight.

### **Trimming of wax blocks**

Excess paraffin was trimmed away leaving at least 5 mm of paraffin on all sides of the tissue. Paraffin block was mounted onto a chuck and finely trimmed leaving 2mm. of paraffin around the specimen on all sides.

### **Section cutting**

Seven (7) microns thick serial sections were cut with the help of rotary microtome machine. Serial sections of the ribbons were kept in order.

### **Mounting the ribbons**

Serial sections were stretched in the tissue floating bath at the temp. of 45°C. Applied the Mayer's albumin adhesive to the clean glass slides and transferred serial sections to the slides in order. Slides were allowed to dry for 8 hours.

### **Dewaxing**

Paraffin was removed from the sections using xylene because paraffin prevents the staining of the tissues.

Xylene	2 minutes
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### **Hydration**

The tissue sections were then passed through descending strengths of ethyl alcohol.

Absolute Ethyl Alcohol	5 minutes
Ethyl Alcohol 90 %	5 minutes
Ethyl Alcohol 70 %	5 minutes
Tap water	1 minute
Distilled water	4 dips

### **Staining**

Slides were stained using Hematoxylin and Eosin (H & E). The sections were immersed in Haematoxylin, to stain the nuclei, for 2 minutes. The tissue sections were washed in running water for 5 minutes. The sections were then passed through descending strengths of ethyl alcohol in order to

remove any Hematoxylin from the cytoplasm of cells (Differentiation, Decolorization).

Ethyl Alcohol 70 %	3 minutes
Ethyl Alcohol 90 %	3 minutes

The sections were then immersed in Eosin to stain the cytoplasm red (Counter staining) for 2 minutes. Water was removed by passing the slides through ascending strengths of Ethyl alcohol (Dehydration) because the usual permanent mounting materials are immiscible with water present in the tissue.

Ethyl alcohol 90%	2 minutes
Absolute ethyl alcohol	2 minutes

This step also served to remove any excess eosin from the tissue (Differentiation; Decolorization).

### **Oiling**

The tissues sections were treated with cedar wood oil.

Cedar wood oil	3 dips
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Slides were immersed in xylene for clearing because permanent section must be translucent.

Xylene I	1 dip
Xylene II	1 dips

### **Mounting cover slips**

Canada balsam was applied to the slides as a mounting medium. Then cover slip was placed on the slide carefully to avoid the air bubbles. After mounting the cover slips slides were placed on warming tray in dust free environment before observing them under microscope.

## **RESULTS AND DISCUSSION**

Trivalent chromium is considered an essential trace element which is involved in metabolism of fats and carbohydrates in mammals [9]. However, exposure to its high doses can exert deleterious effects [10]. Previous studies have extensively reported the toxic effects of hexavalent chromium in both humans and animal subjects. In the current investigations, hepatotoxic and nephrotoxic effects of passively introduced (through diet) trivalent chromium were evaluated in Swiss Albino mice. Liver and kidneys were monitored for morphological and histological changes induced by chromium toxicity.

All treatment groups showed slight discoloration of liver and kidneys. Histological changes in kidneys were observed at all doses (2.5, 3, 3.5 & 4mg/kg/day for three weeks) including glomerular atrophy and mononuclear cell infiltration. While necrosis of hematopoietic tissue was observed at 3.5mg/kg/day. Whereas, liver cross sections showed both abnormal congestion and dilation of centrilobular veins in all treatment groups as compared to control group. Moreover, Fibrosis in portal area of liver was observed at 3.5mg/kg.

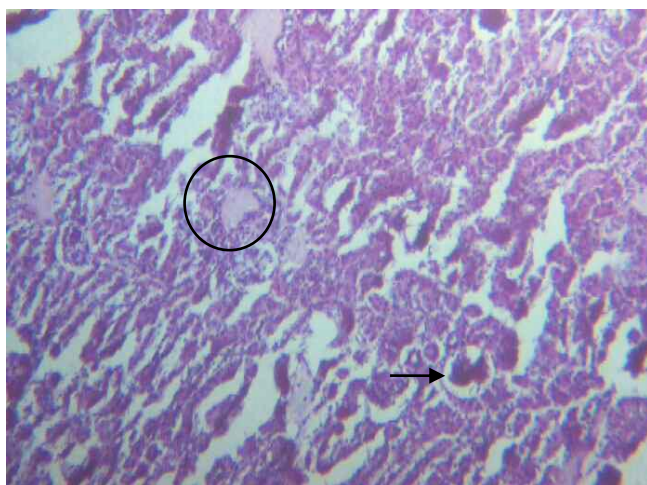


Fig. 1. Glomerular atrophy (arrow) and mononuclear cell infiltration (circle) in kidney cross section

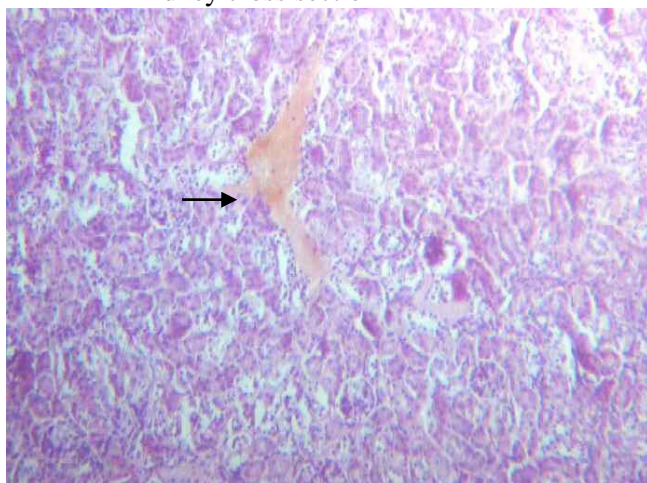


Fig. 2. Necrosis and mononuclear cell infiltration in kidney cross section



Fig. 3. Sinusoid congestion and fibrosis in dilated portal vein in liver cross section

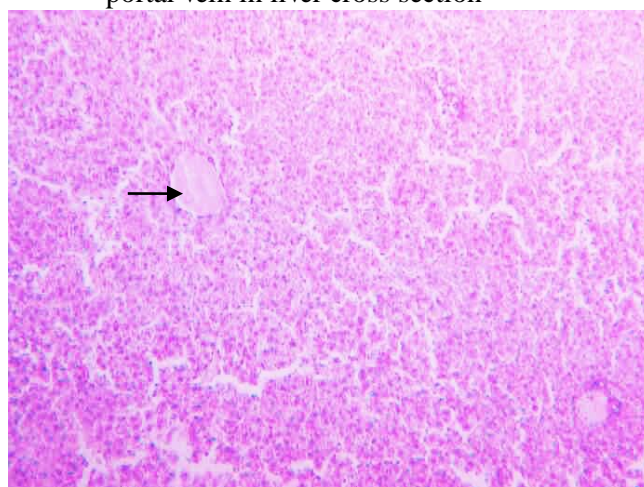


Fig. 4. Normal central vein and sinusoids in liver cross section (control)

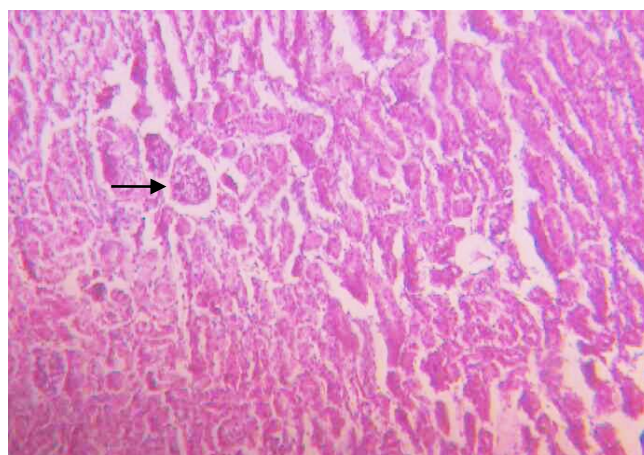


Fig. 5. Normal glomeruli in kidney cross section (control)

These findings are in agreement with previous investigations which report toxic effects of chromium administered through various routes. Another study on rats has also reported the appearance of swollen and edematous hepatocytes, diminished blood sinuses between swollen hepatocytes and vacuolated cytoplasm due to chromium toxicity [11]. It is observed that chromium retention varies in different tissues such as majority of it is accumulated in kidneys as compared to spleen, liver, and gastrocnemius muscles [12]. Thus pathological effects should be more pronounced on kidneys due to higher retention. As current study reveals that more pronounced effects are exerted on kidneys in the form of mononuclear cell infiltration, necrosis and contraction of glomerulus.

In the present study, marked histopathological changes in both liver and kidney were observed upon exposure to high concentrations of chromium sulfate. One of the striking observations is appearance of mononuclear cell infiltrate in kidneys which is not reported in previous investigations. This can be explained by immune-stimulatory effect of chromium. According to Shrivastava et al. [13] chromium can significantly alter the immune response by affecting T and B lymphocytes, macrophages, cytokine production that may induce hypersensitivity reactions.

It may be concluded that like other transition metals toxic effects of trivalent chromium are mediated by the induction of oxidative stress which damages the biological molecules and tissues. Ozawa et al. [14] observed that toxic effects of Cr (III) are mediated by its reduction into Cr (II) in the presence of biological reductants such as L-cysteine and NADH. Free hydroxyl radicals are produced when newly formed Cr (II) reacts with hydrogen peroxide. These hydroxyl radicals in turn induce the tissue damaging effects. Bagchi et al. [15] observed the effects of two dietary Cr (III) supplements i.e. chromium picolinate and niacin bound chromium on mice. The investigations showed that chromium picolinate causes more oxidative stress as compared to niacin bound Cr. The major pathological changes included renal dysfunction, anemia, edematous tissues, production of free radicals and reduction of antioxidant enzymes. In addition, it also induced chromosomal damage and impaired cognitive activity.

The fundamental problem with heavy metals is that although some of them are needed by organisms in trace amounts, when present in excess they cause denaturation of enzymes (Chapman and Reiss, 1999). As Dey et al. [16] found that Cr treatment causes significant decrease in alkaline phosphatase, total ATPase and  $\text{Na}^+\text{-K}^+\text{-ATPase}$  activities of plasma membrane in both liver and kidney. The membrane becomes more permeable with water and glucose entrance in cytoplasm of hepatocytes. Thus these changes in enzyme activities due to Cr (III) might have caused the histological changes in liver and kidney of Albino mice in current experiment. Similar results were observed by Silva et al. [17] who found that trivalent chromium induces cellular alterations such as decrease in membrane cholesterol level and increase in phospholipids level in rat liver. Histological changes were also observed including fibrosis in portal area, congestion of sinusoids, centrilobular veins and portal vessels. Moreover, fibrosis observed in portal area is caused by fibrogenic effects of chromium.

Furthermore, Pan et al. [18] observed that exposure of both hexavalent and trivalent chromium induces hepatotoxicity in female nude mice. Histological examination evaluated the extent of liver damage under different treatments. It was revealed that Cr (VI) compound Potassium dichromate induces greater oxidative stress, apoptosis and hepatotoxicity as compared to Cr (III)- nitrate. Cr affects the activity of several proteins which are involved in carbohydrate metabolism, endoplasmic reticulum stress, calcium homeostasis and apoptosis. Furthermore, expression profiles of cytokeratin were also associated with apoptosis of liver tissues. In another study, Subashini et al. [19] reported that chromium sulfate induces toxicity by affecting plasma electrolytes ( $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Cl}^-$ ) balance and activity of  $\text{Na}^+$ ,  $\text{K}^+$  ATPase in *Cyprinus carpio*. Throughout the experimental period, plasma sodium level was elevated, whereas plasma chloride level was reduced. Cr toxicity in living systems is indicated though altered plasma electrolytes level and ATPase activity.

#### CONCLUSION AND RECOMMENDATIONS

Pathological effects observed in this study suggest that high concentration of trivalent chromium participate in alterations of kidney and liver structure. This study also gives a new evidence for immunostimulatory effects of trivalent chromium as

showed by infiltration of mononuclear cells in kidneys. Furthermore, it strengthens the hypothesis that even passive environmental exposure to chromium may lead to severe health problems. It is basic research to evaluate the side effects of chromium ingestion through food however evaluation of oxidative stress through liver and antioxidant enzyme activity may further provide more sound justification.

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