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Protective Role of Alpha-Tocopherol in Chromium Induced Toxicity in Albino Rat's Liver: A Histopathological Study

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ABSTRACT

Background: To study the effect of chromium on liver and investigate whether alpha-tocopherol could protect the liver from the histological changes induced by hexavalent -Chromium(CrVI). **Methods:** The study was conducted in the department of anatomy in collaboration with department of pharmacology and pathology of Subharti Medical College, Meerut,U.P. The study was done on 36 male adult albino Rats, aged about 60 days with an average body weight of 140+20gm.The chemicals used were Chromium (Cr) as potassium dichromate ($K_2Cr_2O_7$) which was dissolved in distilled water to form a stock solution of 10 mg/ kg body weight. Alfa-tocopherol (Vitamin E) was given in concentration of 125 mg/kg body weight. **Results:** Under light microscopy, the haematoxylin and eosin(H&E) stained section of liver of all the groups were seen. 30 non-overlapping fields per slides were examined to observe the histopathological changes in each group. Statiaticalanaysis was done by SPSS 21 software version. **Conclusion:** The level of significance of all the changes were calculated by Z-proportion test. The $p<0.05$ was considered as statistically significant.

Keywords: Potassium-dicromate, Alpha-tocopherol, Hepatocytes, Sinusoids, Toxicity.

INTRODUCTION

Heavy metals required in minimal amount for normal functioning of body system. Excessive level of the heavy metal example chromium cause damage as well as toxicity to the organism. Toxicity can be manifested in various forms like mental and physical dysfunctions, change in blood composition, pathological changes in vital organs. Long term effect on exposure may result in slow progressive physical, muscular, and neurological degenerative changes.[1]

Chromium is a chemical element with symbol Cr, It is a steely-gray, lustrous, hard and brittle metal which takes a high polish, resists tarnishing and has a high melting point.[2] Chromium metal are commercially produce from chromite, by leaching processes.[3] Chromiumhave proven of high value Due to its highlcorrosive nature, resistance and hardness property. In larger amount and in different forms, chromium can be toxic and carcinogenic. Cr(VI) compounds have been reported to be moretoxic and carcinogenic than those of Cr(III) because the former can pass through cell membranes more easily than the latter and generate oxygen species (ROS), which is an important characteristic of Cr (VI) metabolism4. An excessive quantity of ROScan cause injury to proteins, lipids, and DNA, leading to a state known as cellular oxidative stress.[5] The most important damages caused by extraneous Cr (VI) is the massive ROS production during Cr (VI) reduction in the cell. Liver is the major organ responsible for metabolism, detoxification and secretory functions in the body. Regulates various important metabolic functions in mammalian systems Cr (VI) has been reported to cause hepatotoxicity in humans and laboratory animals primarily through an oxidative stress-mediated mechanism6. Exposure of rats of Chromium induces oxidative stress with an increase in hepatic malondialdehyde (MDA) levels and a decrease in glutathione reductase (GSH) levels.[7] With a decrease of liver glycogen concentration and oxidative denaturation of lipids and proteins.[8] Many studies have

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reported the antioxidant protective effect of vitamin E againstseveral metals inducedhepato-toxicity.The protective effect of vitamin E against Cr-induced hepatotoxicity has been reported, where the authors investigated the direct protective effect of vitamin E on Cr(VI)-induced cytotoxicity and lipid peroxidation in primary cultures of rat hepatocytes.[9] Protective effects of Vitamin E on Liver in animals 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.[10]

The present study was conducted on liver of albino rats, to see the histologicalchanges in liver due to chromium (VI) toxicity and to assess the protective effectsof alpha-tocopherol (vitamin-E) against Cr-induced hepatic injury, oxidative stress, and structural changes in rats when the drugs chromium (VI) and alpha-tocopherol (Vitamin E) were given by the oral routes.

MATERIALS AND METHODS

The present experimental study was conducted in the department of anatomy in collaboration with department of pharmacology and pathology of Subharti Medical College, Meerut,U.P.

- Prior permission was taken from IAEC.
- All experiments were conducted according to CPSSEA guide.
- The chemicals used were of analytical grade. Chromium (Cr) as potassium dichromate (K2Cr2O7) which was dissolved in distilled water to form a stock solution of 10 mg/ kg body weight, and diluted further in sterile saline according to the dose required by rat.
- Alfa-tocopherol(Vitamin E) was given in concentration of 125 mg/kg body weight .
- The study was done on 36 male adult albino Rats, aged about 60 days with an average body weight of 140+20gm.The animals were kept in Polypropylene cage, fed pellet diet and water orally.
- Animals were divided into four groups
- Group 1:- Control group - comprising of 9animals -were given distilled water only.
- Group 2:- Comprising of 9 animals were used for the study of chromium toxicity.
- Group 3:- Comprising of 9 animals were used for the study of effect of chromium along withalpha-tocopherol.
- Group 4:- Comprising of 9animal were used for the study of effect of alpha- tocopherol only.

Groups 2 & 3 were further divided into sub groups as-

a. Acute- Drugs were given for 1 day.
b. Sub-acute-Drugs were given for 14 days .
c. Chronic-Drugs were given for 42 days

Dose of potassium dichromate given was -10 mg/kg body weight.

Dose of alpha-tocopherol given was as -125 mg/kg body weight.

Procedure

Following experimental procedures were adopted for all the four groups. Experimental animals were given chromium as potassium dichromate (K2Cr2O7) dissolved in distilled water at 10mg/kg body weight by oral route.

Group 1- was given distilled water only.

Group 2- 2a) Acute- In this group 10 mg/kg body weight of potassium dichromate dissolved in distilled water was given as single dose orally.

2b) Sub-Acute-In this group 10 mg/kg body weight of potassium dichromate dissolved in distilled water was given daily for 14 days by oral gavage.

2c) Chronic-In this group 10 mg/kg body weight of potassium dichromate dissolved in distilled water wasgiven daily for 42 days by oral gavage.

Group 3-were given 10 mg/kg body weight of potassium dichromate dissolved in distilled water along with alpha-tocopherol 125 mg/kg body weight orally,and in 3 sub-groups.

3a) Acute- drugs were given for 1 day

3b) Sub-Acute- drugs were given for 14 days

3c) Chronic- drugs were given for 42 days.

Group 4- Was maintained as alpha-tocopherol control, and were given alpha-tocopherol,125 mg/kg body weight daily for 14 days by oral gavage.

The animals were sacrificed by stroking the dorsal aspect of the head within 24 hours of the last consumed dose of the drug chromium and alpha-tocopherol51. Thereafter the livers were kept in 10% buffered formaline for preservation , then prepared and stained with haematoxylin and eosin stains , mounted in DPX and observed under microscope.

RESULTS

Histopathological observation: Under light microscopy, the haematoxylin and eosin (H&E) stained section of liver of all the groups were seen. 30 non-overlapping fields per slides were examined to observe the histopathological changes in each group. Statiaticalanaysis was done by SPSS 21 software version. The level of significance of all the changes were calculated by Z- proportion test. The $p<0.05$ was considered as statistically significant.

Control group

Oncomparing several hepaticlobule – the hepatic parenchyma of control group had shown normal

architecture. The hepatocyte appear polyhedral without vacuolated cytoplasm containing basophilic granules and central rounded nucleus [Figure 1].

Cr (VI) toxicity- This group of rats received only potassium dichromate. The group is further sub-divided into three sub-groups in which the drug was administered for – acute (1 day), sub-acute (14 days).

Acute

Showed normal architecture of liver parenchyma. Sub-acute-Showed dilated hepatocytes and dilated sinusoids [Figure 2].

Chronic

Chromium administered rat's liver histology shows congested blood vessels with distorted endothelial lining of sinusoids withdistorted hepatic cells [Figure 3].

Cr (VI) + alpha-tocopherol group-showed that the histopathological changes induced in hepatocytes by chromium toxicity got reduce almost to normal pattern when treated along with alpha-tocopherol [Figure 4].

Alpha

Tocopherol control-The alpha-tocopherol groups of rats showed normal histo-architecture array of hepatic parenchyma.

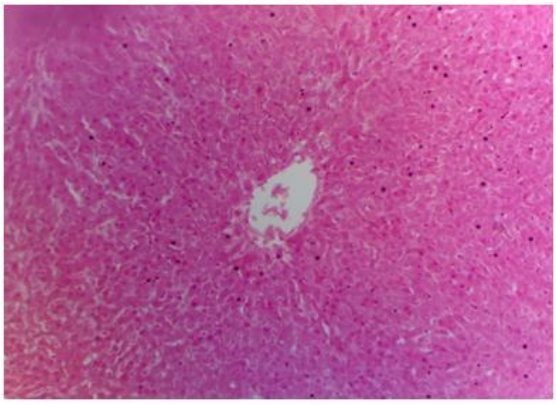


Figure 1: Microphotograph showing H&E stained slide of liver –control group (100X)

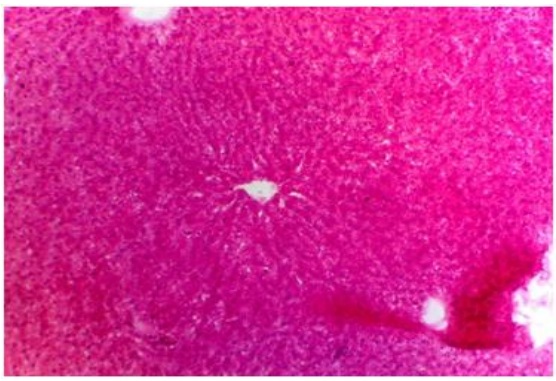


Figure 2: Microphotograph showing H&E stained slide of liver –Cr toxicity group (sub-acute) (100X)

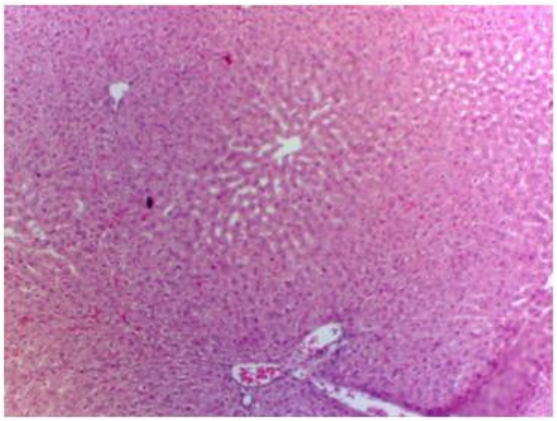


Figure 3: Microphotograph showing H&E stained slide of liver –Cr toxicity group (chronic) (100X)

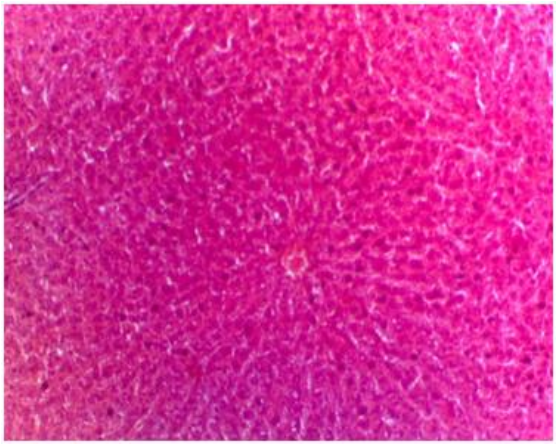
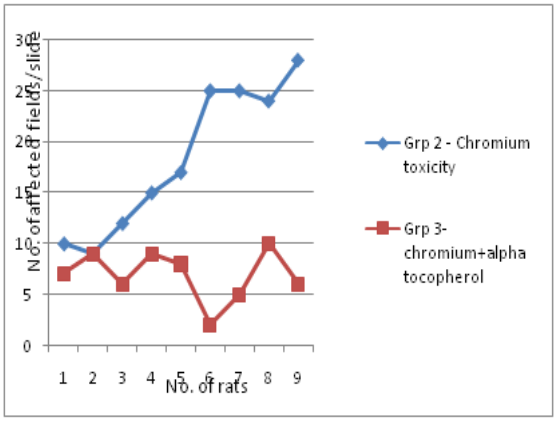


Figure 4: Microphotograph showing H&E stained slide of liver –Cr +alpha-tocopherolgroup (100X)

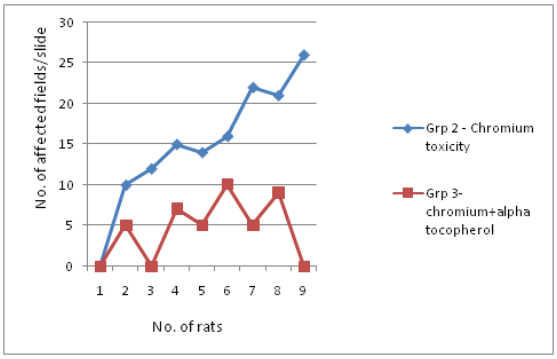
Table 1: Comparing different histological parameters between the 2 groups, by Z-proportion test.

Findings	Group-2, Cr-toxicity (%)	Group3, Cr+alpha-tocopherol (%)	Difference	p-Value
Distorted hepatocytes	61.1	23.0	38.1	<0.001
Dilated sinusoids	50.4	15.2	35.2	<0.001

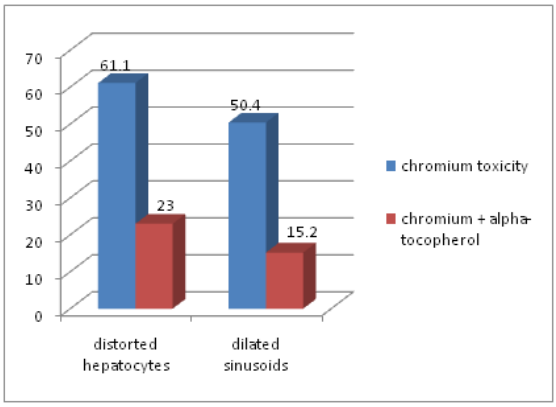
p-value< 0.001 considered extremely significant.



Graph 1: Shows comparison of distorted hepatocytes between 2 groups.



Graph 2: Shows comparison of Dilated Sinusoids between 2 groups.



Graph 3: Shows comparison of different histological parameters between the 2 groups.

DISCUSSION

Significant quantities of toxic metallic elements as chromium are being introduce into our environment from natural and man-made sources.

Chromium at physiological level is involved in regulation of normal carbohydrate metabolism in mammals, so considered to be essential trace element.[11] Trace elements play a part in the synthesis and structural stabilization of both proteins and nucleic acid. They also involve in membrane transport, nerve conduction and muscle contraction.

The entry route of chromium into human body are inhalation, ingestion and dermal absorption. Occupational exposure generally occur through inhalation and dermal contact, where as general population is expose most often by ingestion through chromium content in soil, food and water.

Rate of chromium uptake from the gastrointestinal tract are relatively low and depends on number of factors as- Valency state, (hexavalent chromium being more readily absorb than trivalent-chromium),Water solubility, Gastro-intestinal transit time.[12]

After entering the body trivalent chromium binds to transferring (an iron-transporting protein in plasma) in contrast to hexavalent chromium is readily taken up by erythrocytes, trivalent chromium is widely distributed in the body and accounts for most of the chromium in plasma or tissue but lower cellular permeability compared with hexavalent chromium, was found to play prime role in cellular damage .

The estimated safe and adequate daily dietry intake for trivalent chromium is between 50-200microgm/day. Food grinding and using stainless steel equipments may increase chromium content of food.[13] The over dose of hexavalent chromium compound is considered highly toxic, and are widely recognize as human carcinogen.[14]

It is suggested that hexavalent chromium causes increase risk of bone, prostatic lymphomas reflecting the ability of hexavalent chromium to penetrate all the tissues of the body.[15]

Previous studies of O'Brien TJ have shown that dichromate exposure increases the concentration of reactive oxygen species,which is reflected by damage and distortion of hepatocytes. In our present study too distorted hepatic architecture is seen.

Pattola AK etal5, stated that increase concentration of reactive oxygen species lead to oxidative damage of hepatocytes, reflected by altered histoarchitecture changes and dilatation of sinusoids. Similarly in our present study damage of liver cells occur, with dilatation of sinusoids and distortion of hepatocytes which further leads to degenerative changes.

Da Silva etal in his study of trivalent chromium found damage and distortion of liver hepatocytes.[17] Inpresent study the toxic effect of chromium led to damage of hepatocytes, dilatation of sinusoids. Further in our study it was also seen that when alpha-tocopherol was given there were reversal of histological alteration in liver cell which is similar to finding of Da Silva etal.

Soudani et al,[17] had shown that antioxidant supplement could protect the chromium induce liver tissue damage which in our present study was shown by supplementation of alpha-tocopherol along with chromium, where the reversal of the distorted changes of the liver cell occurred to large extent.

Bursell. SE.et al,[18] in his study proved that alpha-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 our study too liver cells treated withalpha- tocopherol along with hexavalent chromium reverses the altered histological changes of liver cells. Hence alpha-tocopherol (vitamin-E), proved to be hepatoprotective against metal induce toxicity.

So, inpresent study, co-administration of oral alpha- tocopherol along with hexavalent chromium exhibit significant protective effect fromreversal of histological alteration of hepatocytes and sinusoids

of liver and hence restoring the architecture of liver significantly.

CONCLUSION

Hexavalent chromium is the most toxic metal ion, cause adverse health effects following inhalation, ingestion or dermal exposure as , allergic reaction, gastro-enteritis and hepato-cellular deficiencies.

Vitamin E is an essential nutritional element which has biological anti-oxidant role maintain the function of intracellular organelles and cellular membrane integrity, alpha-tocopherol supplementation to chromium fed rats exhibit to reverse effects indicating its protective antioxidant property.

Thus, our present study, confirmed the role of alpha-tocopherol as a scavenger of free radical and proved protective against hexavalent chromium induced toxicity.

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