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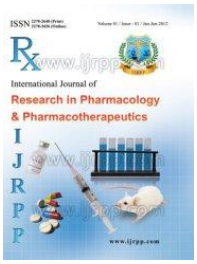

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Research Article ***Open Access***

Protective effect of *Cardiospermum halicacabum* on potassium dichromate induced nephrotoxicity in rats

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ABSTRACT

Potassium dichromate is a chemical compound widely used in metallurgy, chrome plating, chemical industry, textile manufacture, wood preservation, photography, photoengraving, refractory, stainless steel industries and cooling systems. Potassium dichromate a compound is the most toxic form of cr (VI) and has been demonstrated to induce nephrotoxicity associated with oxidative stress in humans and animals.

Aim

The present study was aimed to investigate the protective effect of *Cardiospermum halicacabum* against potassium dichromate induced nephrotoxicity in rats.

Materials and Methods

Rats were divided into four groups of 6 animals each. Group III & group IV received methanolic extract of *Cardiospermum halicacabum* (100mg/kg, 200mg/kg b.w respectively) consecutively for 5 days. Group 1 (control) received normal saline only. Group II (toxic) received vehicle for 5 days. A single dose of $K_2cr_2o_7$ (20mg/kg) was administered subcutaneously on 4th day to all the animals except group 1. The protective effects of *Cardiospermum halicacabum* on $K_2cr_2o_7$ induced nephrotoxicity was investigated by assaying oxidative stress biomarkers, lipid peroxidation, kidney toxicity markers and by histopathological examination of kidney.

Results and Conclusion

Cardiospermum halicacabum pretreatment prevented toxic effects induced by $K_2cr_2o_7$ through a protective mechanism that involved reduction of oxidative stress as well as by restoration of histopathological change against $K_2cr_2o_7$ administration. In conclusion, the results of the present study showed that the methanolic extract of *Cardiospermum halicacabum* has protective effect against potassium dichromate induced nephrotoxicity.

Keywords: $K_2cr_2o_7$, *cardiospermum halicacabum*, Nephroprotective

INTRODUCTION

Chromium is a naturally occurring element found in volcanic dust, in earth crust and is widely distributed in air, water, rocks, soil, plants and animals.(1) The oxidation state and solubility of chromium (cr) compounds determine their toxicity.

In contrast to cr (III), which is a naturally occurring form and an essential trace element for humans and other mammals.[2] Cr(VI) compounds are highly toxic.[3] The hexavalent form is usually linked with oxygen and is a strong oxidizing agent. Studies have shown that cr(VI) is a mutagenic, carcinogenic and teratogenic agent, which easily penetrates the cell membrane in the form of chromate anion.[4] Potassium dichromate, a cr(VI) compound widely used in metallurgy, chrome plating, chemical industry, textile manufacture, wood preservation, photography and photoengraving, refractory and stainless steel industries and cooling systems.[5] There are three possible routes inhalation, dermal contact and ingestion through which exposure to cr(VI) compounds occurs and has been associated with skin, kidney and liver toxicities.[6] Cr(VI) is reduced through reactive intermediates like cr(IV) and cr(V) to the kinetically further more stable cr(III) by intracellular reductants together with glutathione, vitamin c and NADPH-dependent flavo enzymes. It has been demonstrated through in vivo and in vitro studies that the generation of reactive oxygen species occurs through this reduction process due to which various types of cellular damage occurs [7-10]. The kidney is the principal route of cr excretion and it has been reported that acute exposure induces an increase in cr in kidney content on $k_2cr_2o_7$- treated rats. Exposition to cr (VI) produced anatomical lesions at the level of the proximal tubular cells and lipid peroxidation in human kidney. To our knowledge, protective effect of *Cardiospermum halicacabum* on $k_2cr_2o_7$-induced nephrotoxicity has not been explored. Herbal plants have been used for the cure of several human diseases and are gaining more attention due to less toxicity and high efficacy. *Cardiospermum halicacabum* has been reported to be a rich source of β-sitosterol, stigmasterol, flavones alkaloids, steroids, terpenoids, saponins, sugars, essential oil, resin, tannin.

MATERIALS AND METHODS

Chemicals And Plant

$k_2cr_2o_7$ (Potassium dichromate) was purchased from sigma Aldrich company. *Cardiospermum halicacabum* was obtained from plant farm and surroundings of the Raghavendra Institute of Pharmaceutical Education and Research (RIPER). The other chemicals used were in analytical grade.

Plant Extraction Process

The whole plant of *Cardiospermum halicacabum* was collected and shade dried, powdered to get coarse powder. Powder was extracted with methanol at 60-70^{0}c by continuous hot percolation using soxhlet apparatus. Then extract was concentrated in the desiccators using anhydrous lime as a dehydrating agent. Black viscous residue was obtained with methanol extraction. The residue was stored at 4^0 c until use.

Animals

Male wistar rats (120-150g) were obtained from the Raghavendra enterprises, Bangalore, India and were housed in a ventilated room at 25±5^0 c under 12 h light/dark cycle. The animals were acclimatized for one week before the study and had free access to standard laboratory feed and water ad libitum. The study was approved by the institutional animal ethical committee.

Experimental Design

Rats were divided into four groups of 6 animals each. Methanolic extract of *Cardiospermum halicacabum* was administered in the form of suspension using 1% cmc as a suspending agent in distilled water.

Animals of group I (control) received normal saline only.

Animals of group II (toxic) received vehicle (10mg/kg/day) for 5 days.

Animals of group III received (100mg/kg b.w) of Methanolic extract of *Cardiospermum halicacabum* for 5 days.

Animals of group IV received (200mg/kg b.w) of Methanolic extract of *Cardiospermum halicacabum* for 5 days.

A single dose of $K_2cr_2o_7$ (20mg/kg) was administered subcutaneously on 4th day to all the animals except group I.

On 6th day, blood samples were withdrawn from retro-orbital venous plexus and serum was separated for the estimation of biochemical parameters. Animals were sacrificed and their kidneys were isolated for histopathological studies.

Biochemical Assays

Sera samples were collected to determine urea, creatinine in semi auto-analyser using commercially available kits. Superoxide dismutase activity was determined by the method of Misra and Fridovich (1967). Catalase activity was

determined by the method of Hugo E. Aebi (1974). Reduced glutathione was determined by the method of Ellman. Lipid peroxidation (LPO) was measured in terms of malondialdehyde (MDA).

Histopathological examination

The kidneys were quickly removed after sacrifice of rats and were fixed in 10% neutral buffered formalin solution for histopathological processing. Sections were stained with haematoxyline and eosin before being observed under an Olympus microscope at x 400 magnification.

Statistical analysis

Results were expressed as mean±SEM and differences between the groups were determined by ANOVA (One Way Analysis of Variance) followed by Dunnetts multiple comparison test. $P<0.05$ was considered to be significant.

RESULTS

Table 1: Results of Treatment of Methanolic Extract of *Cardiospermum halicacabum* on SOD, Catalase, GSH, and LPO on Administration of $K_2Cr_2O_7$ in Kidney of Rats

Treatment regimen per group	Superoxide dismutase (U/mg protein)	Catalase (µ mol of H_20_2 decomposed/min/mg protein)	Reduced glutathione (µg/g tissue)	Lipid peroxidation (µ mol MDA/ mg protein)
NC	34.17±0.60	57.83±1.16	71.20±1.66	15.30±1.26
TC ($K_2Cr_2O_7$)	12.67±1.25**	18.33±0.88**	28.30±0.66**	68.00±1.46**
MEC D1 + $K_2Cr_2O_7$	27.17±0.79$^{\#\#}$	41.17±2.58$^{\#\#}$	49.70±2.95$^{\#\#}$	23.30±1.52$^{\#\#}$
MEC D2 + $K_2Cr_2O_7$	31.17±0.60$^{\#\#}$	49.00±1.18$^{\#\#}$	66.00±1.88$^{\#\#}$	15.20±0.94$^{\#\#}$

Results represent mean±SEM of six animals per group. Results obtained are significantly different from control group ($^{**}P<0.001$). Results obtained are significantly different from $K_2Cr_2O_7$ treated group ($^{\#\#}P<0.001$). D1 = 100mg/kg b wt; D2 = 200mg/kg b wt.

Table 2: Results of Treatment of Methanolic Extract of *Cardiospermum halicacabum* on Serum Urea and Serum Creatinine

Treatment regimen per Group	Serum urea (mg/dl)	Serum creatinine (mg/dl)
NC	7.70±0.55	0.17±0.006
TC ($K_2Cr_2O_7$)	11.5±0.64**	0.40±0.09*
MEC D1 + $K_2Cr_2O_7$	8.43±0.43$^{\#\#}$	0.11±0.02$^{\#\#}$
MEC D2 + $K_2Cr_2O_7$	8.98±0.28$^{\#}$	0.13±0.009$^{\#\#}$

Results represent mean±SEM of six animals per group. Results obtained are significantly different from control group ($^{**}P<0.001$). Results obtained are significantly different from $K_2Cr_2O_7$ treated group ($^{\#}P<0.05$) ($^{\#\#}P<0.001$). D1 = 100mg/kg b wt; D2 = 200mg/kg b wt.

Effect of methanolic extract of *Cardiospermum halicacabum* on kidney antioxidants

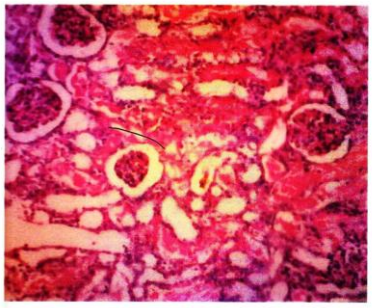
Significant ($p<0.01$) increase in lipid peroxidation and significant ($p<0.01$) decrease in superoxide dismutase, Catalase, reduced glutathione was observed in toxic control animals when compared to normal control animals. Treatment with methanolic extract of *Cardiospermum halicacabum* at doses (100mg/kg, 200mg/kg b wt) significantly ($p<0.01$) decreases the lipid peroxidation whereas significantly ($p<0.01$) increase the superoxide dismutase, Catalase, reduced glutathione when compared to toxic control animals.

Effect of methanolic extract of *Cardiospermum halicacabum* on serum urea and creatinine

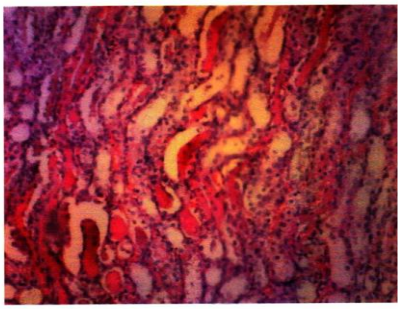
Significant ($p<0.01$) increase in serum urea, creatinine levels were observed in toxic control animals when compared to normal control animals.

Treatment with methanolic extract of *Cardiospermum halicacabum* at doses (100mg/kg, 200mg/kg b wt) significantly ($p<0.01$) decreases the serum urea and creatinine levels when compared to toxic control animals in a dose dependent manner.

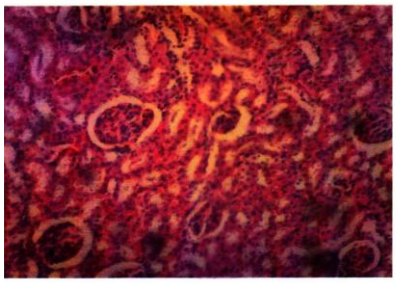
HISTOPATHOLOGY RESULTS



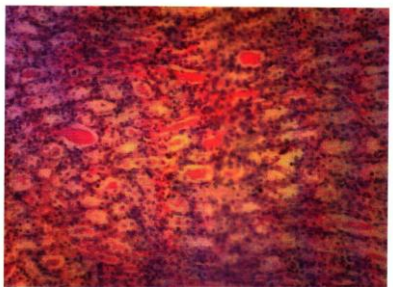
Normal kidney



Potassium dichromate treated kidney



Methanolic extract of cardiospermum halicacabum treated kidney (100mg/kg)



Methanolic extract of cardiospermum halicacabum treated kidney (200mg/kg)

Fig.1: Kidney histology of results

Kidney of normal rats showed normal histological architecture. Kidney of potassium dichromate treated rats showed degenerative changes, Glomerular nephritis and significant damage in tubular epithelial cells. Kidney of methanolic extract of *Cardiospermum halicacabum* treated rats (100mg/kg) showed mild inflammation in both cortical & medullary region. Kidney of methanolic extract of *Cardiospermum halicacabum* treated rats (200mg/kg) showed regenerative appearances, hence it is similar to normal appearance.

DISCUSSION

The effect of toxic metals on the kidney has been known for many years and is one of the most common kidney problems and occurs when body is exposed to a drug or toxin [(11)]. The kidney is the main route of Cr excretion, it has been reported that acute exposure to $K_2cr_2o_7$ in rats induced an increase in kidney chromium content [(12)]. Cr (VI) compounds are easily taken up by the cells and are subsequently reduced to Cr (III) species. This reduction generates free radicals, which play a major role in the adverse biological effects [(13)]. Previous study showed that exposure to Cr (VI) compounds can lead to nephrotoxicity in humans and experimental animals [(14)]. The role of oxidative stress in dichromate-induced kidney damage has been supported by the present work and previous studies. In the present study, potassium dichromate treatment caused nephrotoxicity as evidenced by marked elevation in blood urea and creatinine. In the present study $K_2cr_2o_7$ treatment alone raised renal lipid peroxidation and exhausted renal reduced glutathione, catalase, and superoxide dismutase as compared to their vehicle treated

control. However, coadministration of methanolic extract of *Cardiospermum halicacabum* (P.O) with $K_2cr_2o_7$ (S.C.) dose dependently decreased the lipid peroxidation and enhanced the reduced glutathione, catalase, superoxide dismutase.

CONCLUSION

In the present study potassium dichromate induced rats showed significant decrease in superoxide dismutase, catalase, reduced glutathione whereas significant increase in lipid peroxidation when compared to normal control animals. Potassium dichromate induced rats also showed significant increase in serum urea and creatinine when compared to normal control animals. Pretreatment with methanolic extract of *Cardiospermum halicacabum* at doses of 100 mg/kg, 200mg/kg bw restored the anti-oxidant parameters and normalise the serum urea, creatinine levels significantly when compared to the $K_2cr_2o_7$ treated rats. In conclusion methanolic extract of *Cardiospermum halicacabum* ameliorated the $K_2cr_2o_7$ induced nephrotoxicity in rats.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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