ORIGINAL ARTICLE

ANTIOXIDANT EFFECT OF VITAMIN E ON CARBON TETRACHLORIDE INDUCED TUBULOINTERSTITIAL AND GLOMERULAR DAMAGE IN THE KIDNEYS OF ALBINO MICE

Shabnum Aamir, Zia ud Din, Zahid Sarfaraz Khan*, Humaira Imtiaz, Faheem ul Haq***, Hajira Ishaq†**

Department of Anatomy, Khyber Medical College, Peshawar, *Khyber Girls Medical College, Peshawar, ** Department of Anatomy, ***Department of Urology Ayub Medical College, Abbottabad, †Department of Biochemistry, Khyber Medical College-Pakistan

Background: Chemical induced nephrotoxicity is one of the main causes of acute kidney injury. The objective of this study was to determine the antioxidant effect of vitamin E against carbon tetrachloride induced tubulointerstitial and glomerular damage in the kidney of albino mice. **Methods:** The study had been conducted on albino mice. The duration of study was for five weeks. A total of 35 animals were randomly divided into five groups A, B, C, D and E .The group A served as control group, group B was administered only with carbon tetrachloride (no vitamin E) and groups C, D and E received test drug (vitamin E) in doses of 1, 10 and 50mg/kg body weight respectively along with CCl_4. The animals were dissected and kidneys were excised for microscopic study for possible histo-morphological effects. **Results:** It was observed that carbon tetrachloride treated experimental groups developed tubulo-interstitial and glomerular changes as compared to control group A. The results suggested that these changes were significantly reduced in vitamin E treated groups especially in dose of 50 mg/kg body weight. **Conclusion:** This study reveals that tubulo-interstitial and glomerular damage caused by carbon tetrachloride can be reduced by vitamin E in dose of 50 mg/kg body weight.

Keywords: Vitamin E; Carbon tetrachloride and Glomerular damage

Citation: Aamir S, Ziauddin, Khan ZS, Imtiaz H, Haq F, Ishaq H. Antioxidant effect of vitamin e on carbon tetrachloride induced tubulointerstitial and glomerular damage in the kidneys of albino mice. J Ayub Med Coll Abbottabad 2020;32(3):295–8.

INTRODUCTION

Chemical and drug induced nephrotoxicity is one of the chief causes of acute kidney injury which could be due to inflammation, tubular cell toxicity or may be due to disturbance in intraglomerular hemodynamic. It is accepted as main causes of mortality and morbidity.[1] Various drugs like non-steroidal anti-inflammatory drugs (NSAIDS), chemotherapeutic agents and aminoglycosides are the leading cause of renal damage.[2,3] Many chemicals such as sodium oxalate, ethylene glycol and some heavy metals like arsenic and mercury have also toxic effect on kidneys.

Carbon tetrachloride (CCl_4) is a well-recognized nephrotoxic.[4–6] CCl_4 has been reported with several hazardous effects on environment; ozone depletion is being the most common. Beside environmental hazards it has also been associated with the variety of human health risks. It has been documented that the liver and kidney are not the only target organs affected by CCl_4, since it also causes fatal outcomes in other tissues such as lungs, testis, blood and brain.[7–9] CCl_4 like other toxic chemicals causes oxidative stress by generating free radicals in the tissue.[10] Histological studies revealed that CCl_4 causes the degenerative changes in kidney such as swelling of renal tubules, vacuolation and atrophy of glomeruli.[11]

Recognition of the disease process at its earlier stage and removal of the causative agent is the only usual significant therapy.[12] Nephrotoxicity can be reduced with certain compound called nephroprotective agents. Many studies supported the role of antioxidants in combating the oxidative damages caused by CCl_4 by enhancing the antioxidant enzymes level in the body such as catalase and superoxide dismutase thus restoring the biological functions of the cells. Recently both in vitro and in vivo trials (using animal models) have been conducted to assess the role of various substances as nephroprotective agents. Among in vivo studies the carbon tetrachloride induced nephrotoxic models are generally used.[13]

Previous studies have shown that antioxidants like N-acetyl cysteine, silymarin and vitamin E partially improve tissue injuries. In present study the vitamin E has been used to demonstrate its effectiveness in reducing the

tubulo- interstitial and glomerular damage induced by CCl_4 in animal model.

MATERIAL AND METHODS

The experiment was performed on male BALB C mice (20–30 g). Animals were kept in well ventilated animal house with 12 hr light and dark photo cycle. They were fed on standard laboratory food. The general behaviour and body weight of animals was recorded regularly throughout the experimental period. The animals were randomly grouped in to group A (control group), group B (administered with CCl_4) and group C, group D and group E.

The group B, C and E received vitamin E orally in dose of 1, 10 and 50mg/kg body weight respectively for the duration of 5 weeks. The CCl_4 nephrotoxicity were induced in mice at a dose of 1 ml/kg subcutaneously weekly. The CCl_4 was mixed with olive oil. At the end of experiment all animals were weighed again before they were sacrificed. The kidneys were excised and gross examination was done.

Finally, specimens were preserved in separate containers in 10% formalin. Tissues were stained with Hematoxylin and Eosin and were studied with light microscope for histological changes. The changes were noted at magnification of 4×, 10× and 40×. EGTI Scoring and grading system was applied in order to evaluate the degree of nephrotoxicity.[14] The results were expressed as mean and standard deviation of variables. One-way ANOVA was performed and the level of significance was set at p-value <0.05.

RESULTS

The CCl_4 treated animals were observed with changes in behaviour immediately after administration of CCl_4 injection. The animals appeared less active and lethargic. Moreover, the change in colour of kidneys was noticed in animals exposed to CCl_4. They were lustreless and pale brown in colour.

The tissues were examined under light microscope for following parameters.

i) Tubulo-interatitial damage:

These changes were graded as no damage = 0, inflamation and heamorrhage in less than 25% =1, nesrosis up to 25% = 2, necrosis upto 60% = 3, necrosis more than 60%= 4. One-way ANOVA shows highly significant p-value (0.0001<0.05). It was observed that CCl_4 treated experimental groups B, C, D and E developed tubulointerstitial changes as compared to control group A. The results are mentioned in table-1.

ii) Glomerular damage:

These changes were graded as no damage = 0, thickening of bowmans capsule =1,retraction of glomerular tuft = 2 and fibrosis = 3. Statistical analysis one-way ANOVA showed highly significant p-value (0.0001 <0.05). The results are shown in table-2.

The microscopic study of the kidneys sections of a control group showed normal parenchymal architecture and no significant lesions were noticed.

The representative photographs are mentioned in figure-1 and 2.

Table-1: Shows vitamin E effect on tubulointerstitial changes

Groups	n	Mean	SD
Group A (Control)	7	0.00	0.000
Group B (CCl_4 only)	7	2.50	0.233
Group C (CCl_4 + 1 mg/kg Vitamin E)	7	2.26	0.1949
Group D (CCl_4 + 10 mg/kg Vitamin E)	7	2.26	0.1949
Group E (CCl_4 + 50 mg/kg Vitamin E)	7	0.8 4	0.307

(p-value =0.0001 <0.05)

Table-2: To show vitamin E effect on glomerular damage

Groups	n	Mean	SD
Group A (Control)	7	0.00	0.000
Group B (CCl_4 only)	7	2.52	0.1303
Group C (CCl_4 + 1 mg/kg vitamin E)	7	2.28	0.1788
Group D (CCl_4+ 10 mg/kg Vitamin E)	7	2.3	0.1224
Group E (CCl_4 + 50 mg/kg Vitamin E)	7	1	0.000

(p-value =0.0001<0.05)

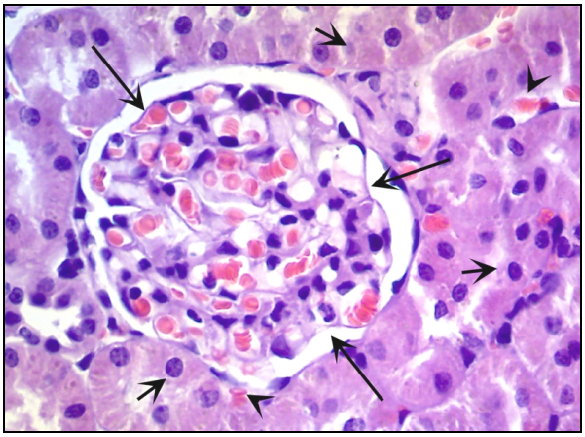

Figure-1: Photomicrograph of a section of kidney of a control group

showing normal histological appearance of glomerulus with normal visceral and parietal layers of the Bowman's capsule (large arrows), cuboidal cells of the proximal convoluted tubules containing a brush border extending into the lumen of the renal tubules (small arrows). The interstitial spaces among the renal tubules have normal appearing red blood cells (arrow heads).

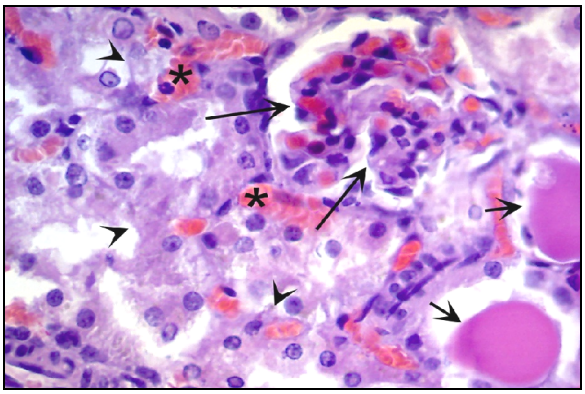

Figure-2: Photomicrograph of a section of kidney from a mouse administered with CCl_4
showing atrophy of the glomerulus (large arrows), tubular necrosis (arrow heads) and cellular cast within lumen of the tubules (small arrows). The interstitial spaces among the tubules are heavily congested with red blood cells.

DISCUSSION

Present study was conducted to demonstrate the protective ability of vitamin E against renal damage induced by CCl_4 in albino mice. The kidneys are one of the most important organs. They remove waste products from the body, maintain balanced electrolyte levels, and regulate blood pressure. Various drugs and chemicals are responsible for nephrotoxicity. Carbon tetrachloride (CCl_4) has been used widely to induce tissue injury as a model to study the protective effects of drugs and plant extracts.[15,16] The carbon tetrachloride leads to formation of free radicals and it reduces activity of antioxidant enzymes.[17] A single dose of CCl_4 leads to oxidative stress and lipid peroxidation, which causes leakage of cellular enzymes in to blood and causes cell damage.

It has been reported by previous studies that CCl_4 causes extensive renal damages. One of the renal histopathological studies on rats conducted by Rincon AR in 1999, demonstrated the glomeruli with mesengial hypercellularity and capillary wall thickening. In some other studies CCl_4 is associated with the significant alteration in renal function tests and histological studies revealed the degenerative changes such as swelling of renal tubules and atrophy of glomeruli.

In the current study CCl_4 was used to induce renal injury. The carbon tetra chloride caused significant renal injury in group B, C, D and group E observed as inflammation and haemorrhage in tubules, tubular necrosis and glomerular damage. The glomerular changes were evident by thickening of Bowman 's capsule and retraction of glomerular tuft. The results suggested that these changes were significantly reduced in vitamin E treated groups especially in dose of 50 mg/kg body weight.

Highly significant work has been done till recently, that clearly demonstrates the potent nephrotoxic effect of carbon tetrachloride.[18] One of the study is recently been conducted by Rashid in 2013 concluded the protective role of flavonoid containing extract of *Oxalis corniculata* in CCl_4 treated animals. Other study performed by Sibel Ozden in 2012 in which animal model of renal damage was designed and protective role of vitamin E was observed in dose of 100mg/kg body weight.[19]

Consequently, the present study findings demonstrate that the vitamin E in a dose of 50 mg/kg body weight showed a remarkable improvement in tubular and glomerular damages in kidneys of mice. The antioxidant ability of vitamin E could justify its protective role.

CONCLUSION

This study reveals that tubulo-interstitial and glomerular damage caused by CCl_4 can be reduced by vitamin E in dose of 50 mg/kg body weight.

AUTHORS' CONTRIBUTION

SA: Concept of main theme, study design, literature search, write-up. ZUD, ZS & HI: Data collection, analysis and interpretation. FUH & HI: Proof reading and minimising plagiarism.

REFERENCES

1. Shahbazi F, Dashti-Khavidaki S, Khalili H, Lessan-Pezeshki M. Potential renoprotective effects of silymarin against nephrotoxic drugs: a review of literature. J Pharm Pharm Sci 2012;15(1):112–23.
2. Hoitsma AJ, Wetzels JF, Koene RA. Drug induced nephrotoxicity. Aetiology, clinical features and management, Drug Saf 1991;6(2):131–47.
3. Barry M, Brenner FC. Rector. The kidney 6th ed. Vol I, Philadelphia W.B. Saunders, 2000; p.3–67.
4. Ogeturk M, Kus I, Colakoglu N, Zararsiz I, Ilhan N, Sarsilmaz M. Caffeic acid phenethyl ester protects kidneys against carbon tetrachloride toxicity in rats. J Ethnopharmacol 2005;97(2):273–80.
5. Ohta Y, Kongo M, Sasaki E, Nishida K, Ishiguro I. Therapeutic effect of melatonin on carbon tetrachloride-induced acute liver injury in rats. J Pineal Res 2000;28(2):119–26.
6. Bhattacharya H, Lun L, Gomez GD. Biochemical effects to toxicity of CCL4 on rosy barbs (Puntius conchonius) Our Nat 2005;3(1):20–5.
7. Khan MR, Rizvi W, Khan GN, Khan RA, Shaheen S. Carbon tetrachloride-induced nephrotoxicity in rats: Protective role of Digera muricata. J Ethnopharmacol 2009;122(1):91–9.
8. Sreelatha S, Padma PR, Umadevi M. Protective effects of Coriandrum sativum extracts on carbon tetrachloride-induced hepatotoxicity in rats. Food Chem Toxicol 2009;47(4):702–8.
9. Adaramoye O. Comparative effects of vitamin E and kolaviron (a biflavonoid from Garcinia kola) on carbon

tetrachloride-induced renal oxidative damage in mice. Pak J Biol Sci 2009;12(16):1146–51.
10. Venkatanarayana G, Sudhakara G, Sivajyothi P, Indira P. Protective effects of curcumin and vitamin E on carbon tetrachloride-induced nephrotoxicity in rats. EXCLI J 2012;11:641–650.
11. El-kholy TA, Naglaa H, Abbas HY. Protection of the Mushroom (shiitake" Lentinus-edodes) against Carbon-Tetrachloride-Induced Renal Injury in Rats. Life Sci J 2013;10(1):1701–8.
12. Paller MS. Drug induced nephropathies. Med Clin North Am1990;74(4):909–17.
13. Khan RA, Khan MR, Sahreen S. CCl 4-induced hepatotoxicity: protective effect of rutin on p53, CYP2E1 and the antioxidative status in rat. BMC Complement Altern Med 2012;12(1):178.
14. Chavez R, Fraser DJ, Bowen T, Jenkins RH, Nesargikar P, Pino-Chavez G, *et al*. Kidney ischaemia reperfusion injury in the rat: the EGTI scoring system as a valid and reliable tool for histological assessment. J Histol Histopathol 2016;3(1):1–7.
15. Ganie SA, Haq E, Hamid A, Qurishi Y, Mahmood Z, Zargar BA, *et al*. Carbon tetrachloride induced kidney and lung tissue damages and antioxidant activities of the aqueous rhizome extract of Podophyllum hexandrum. BMC Complement Altern Med 2011;11(1):17.
16. Manjrekar AP, Jisha V, Bag PP, Adhikary B, Pai MM, Hegde A, *et al*. Effect of Phyllanthus niruri Linn treatment on liver, kidney and testes in CCl 4 induced hepatotoxic rats. Indian J Exp Biol 2008;46(7):514–20.
17. Burk RF, Lane JM, Patel K. Relationship of oxygen and glutathione in protection against carbon tetrachloride-induced hepatic microsomal lipid peroxidation and covalent binding in the rat. Rationale for the use of hyperbaric oxygen to treat carbon tetrachloride ingestion. J Clin Invest 1984;74(6):1996–2001.
18. Ozturk F, Ucar M, Ozturk IC, Vardi N, Batcioglu K. Carbon tetrachloride-induced nephrotoxicity and protective effect of betaine in Sprague-Dawley rats. Urology 2003;62(2):353–6.
19. Ozden S, Catalgol B, Gezginci-Oktayoglu S, Karatug A, Bolkent S, Alpertunga B. Acute effects of methiocarb on oxidative damage and the protective effects of vitamin E and taurine in the liver and kidney of Wistar rats. Toxicol Ind Health 2013;29(1):60–71.

Submitted: January 28, 2020	*Revised: March 6, 2020*	*Accepted: March 6, 2020*

Address for Correspondence:

Shabnum Aamir, Department of Anatomy, Khyber Medical College, Abbottabad-Pakistan
Email: behram2006@gmail.com