Ameliorative Effects of Alpha-tocopherol in Carboplatin Induced Toxicity on Histomorphology of Renal Cortex in Rats

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ABSTRACT

Objective: To evaluate the histomorphological response of alpha-tocopherol co-administration with carboplatin chemotherapy.

Study Design: A laboratory-based experimental study.

Place and Duration of the Study: Anatomy Department, Army Medical College / National University of Medical Sciences (NUMS), Rawalpindi, Pakistan, from January to December 2021.

Methodology: Thirty adult Sprague-Dawley rats were divided into three groups of ten rats each. Control group A received normal diet and water, experimental group B was administered single injection of carboplatin 2.5 mg/Kg intraperitoneally; and experimental group C along with carboplatin injection also received alpha-tocopherol 62.7 mg/Kg daily. At the end of 12 weeks, the euthanasia of animals was done and kidneys were dissected out. Right-sided kidneys were stained with Haematoxylin and Eosin. Micrometry was done to measure the diameters of renal cortical tubules and renal corpuscles.

Results: The proximal and distal tubular and luminal diameter and transvertical diameter of renal corpuscle were increased in group B as compared to control group A. In group C, the proximal and distal tubular diameters were 5.175 ± 0.39 µm and 3.88 ± 0.364 µm, respectively; proximal and distal luminal diameters were 2.67 ± 0.35 µm and 1.64 ± 0.24 µm, respectively and transvertical diameter of renal corpuscle was 12.16 ± 0.870 µm. These values were less than experimental group B and closer to that of control group A.

Conclusion: Renal microscopic parameters showed improvement in the group administered with alpha-tocopherol. Therefore, alpha-tocopherol has ameliorative effects on carboplatin-induced renal damage.

Key Words: Alpha-tocopherol, Carboplatin, Renal corpuscle, Tubules.

INTRODUCTION

Carboplatin is a platinum-containing chemotherapeutic agent.¹ It has less adverse effects as compared to its parent medicine cisplatin.² Carboplatin performs its functions by Deoxyribonucleic acid (DNA) methylation; and the main compound and its metabolites are excreted from the body through the renal pathway.³

Alpha-tocopherol is a form of vitamin E which is present most widely in nature. It is a potent antioxidant and thus it maintains and regulates the long-chain polyunsaturated fatty acids in cell membranes and is necessary for their function.⁴ It helps in boosting up the overall immunity of the human body, so it is also used as a dietary supplement.⁵

Vitamin E binding proteins are present in biological cells, plasma cells as well as tumour cells.² Alpha-tocopherol acts as a peroxide radical scavenger. It terminates the oxidation reactions and hence protects the human body from reactive oxygen species. In chemotherapy, along with the toxic effects of the cytotoxic drug, reactive oxygen species are produced. The level of antioxidant defence mechanisms in the body also goes down.⁷ So, patients undergoing chemotherapy are given supportive treatment which includes antibiotics, anti-emetics, anti-diarrheals and multivitamins to alleviate the harmful effects of chemotherapy drugs. The multivitamin regime does not contain natural or synthetic forms of vitamin E. There are many studies regarding it but the effect of alpha-tocopherol only on patients taking chemotherapy has not been studied.

When carboplatin is excreted via renal pathway, there is kidney damage as it alters the vascular and cellular mechanisms along with alteration in molecular pathways.⁸ This study was performed to evaluate the histological effects of co-administration of alpha-tocopherol with carboplatin on rat renal cortex. It is evidence-based knowledge for oncologists to develop supportive therapy for cancer patients while undergoing carboplatin chemotherapy.
METHODOLOGY

It was a laboratory-based experimental study, conducted at the Anatomy Department, Army Medical College / National University of Medical Sciences (NUMS), Rawalpindi. Collaboration was done with the National Institute of Health (NIH), Islamabad, Pakistan and the Pathology Department, Army Medical College / NUMS, Rawalpindi, Pakistan. The protocol of the study was approved by the Ethical Review Committee (ERC), Army Medical College, NUMS, Rawalpindi, Pakistan under ERC certificate ERC/ID/128.

Thirty adult Sprague-Dawley rats, aged ten to twelve weeks were selected for the study from the animal house of the National Institute of Health, Islamabad. Their body weight was 250 ± 50 grams. Equal number of male and female rats were selected. Female pregnant rats and rats with any obvious injury and disease were excluded from the study.

They were kept at standard temperature 21 ± 2 degree Celsius in a room maintained on a 12-hour light/dark cycle and in standard humidity conditions.

The animals were divided into three groups on a random basis, each group having ten rats. All groups had equal numbers of male and female rats. To avoid mating, rats of both genders were kept in separate cages. Group A was control group in which rats were given standard diet and water ad libitum for 12 weeks. Group B and C were the experimental groups. Rats of group B were administered a single injection of carboplatin through intraperitoneal route on day 1 of experiment at the dose of 2.5mg/kg along with standard diet and water ad libitum. The rats of group C were administered carboplatin injection and received alpha-tocopherol daily at the dose of 62.7 mg/kg via oral gavage in addition to standard diet and water ad libitum starting from the second day of the experiment till the period of 12 weeks. Carboplatin was procured as 450 mg injection. Alpha-tocopherol was obtained in the form of 200 mg capsules.

At the end of 12 weeks, rats were euthanized by overdose of chloroform. The kidneys were dissected out. After washing with normal saline, the right kidneys were fixed in 10% formalin as the study was performed on the right kidneys. Tissue processing was done and then staining was done with Haematoxylin and Eosin (H&E). Two slides of each specimen were made.

The tissues were processed and stained with H&E. Right kidney was selected randomly out of both kidneys as the organ to be observed for microscopy. Cortex of the right kidney of each slide with renal tubules and renal corpuscle was observed under Olympus light microscope at magnification of 40X. Pictures were taken with Olympus digital camera and Sony 16 mega pixel camera. Readings for tubular diameter and luminal diameter of proximal convoluted tubules (PCT) and distal convoluted tubules (DCT) and transversal diameter of renal corpuscle were measured by micrometry. The eyepiece of microscope was calibrated with the help of a stage micrometer. A 0.25 micrometer calibration factor was obtained for 40X and the number of eyepiece divisions for each reading was multiplied by 0.25. Three random fields were observed in one slide, one from each pole and one from mid portion. For tubule diameter, external diameter from basement membrane of one side of tubule to basement membrane of cells on opposite side was measured.

![Micrographs](image-url)

**Figure 1: Photomicrographs showing renal corpuscle of all three groups at 40X magnification.**
Green arrows point towards the renal corpuscle.
Photomicrograph A showing a histological section of kidneys with the normal structure of renal corpuscle in animal A1.
Photomicrograph B shows distorted renal architecture showing dilated renal corpuscle with increased urinary space and shrunken glomerular capillaries in animal B3.
Photomicrograph C shows improved renal architecture with renal corpuscle in animal C4.

![Micrographs](image-url)

**Figure 2: Photomicrographs showing renal tubules of all three groups at 40X magnification**
Blue arrows point towards proximal convoluted tubules (PCT)
Orange arrows point towards distal convoluted tubules (DCT)
Photomicrograph A showing histological section of kidneys with normal structure of PCT and DCT in animal A3.
Photomicrograph B showing distorted renal architecture with dilated PCT and DCT in animal B6.
Photomicrograph C showing improved renal architecture with rounded tubules and diameter of PCT and DCT close to control in animal C5.
### Table I: Mean values in micrometer (µm) of microscopic parameters of control group A and experimental groups B and C.

<table>
<thead>
<tr>
<th>Parameters (µm)</th>
<th>Group A (n=10)</th>
<th>Group B (n=10)</th>
<th>Group C (n=10)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distal luminal diameter</td>
<td>1.445±0.171</td>
<td>1.772±0.232</td>
<td>1.642±0.246</td>
<td>0.009**</td>
</tr>
<tr>
<td>Distal tubular diameter</td>
<td>3.590±0.296</td>
<td>3.885±0.372</td>
<td>3.880±0.364</td>
<td>0.011*</td>
</tr>
<tr>
<td>Proximal luminal diameter</td>
<td>2.450±0.172</td>
<td>2.877±0.316</td>
<td>2.670±0.356</td>
<td>0.112</td>
</tr>
<tr>
<td>Proximal tubular diameter</td>
<td>4.716±0.303</td>
<td>5.235±0.467</td>
<td>5.175±0.399</td>
<td>0.013*</td>
</tr>
<tr>
<td>Transvertical diameter of renal corpuscle</td>
<td>11.790±0.814</td>
<td>12.222±0.888</td>
<td>12.165±0.870</td>
<td>0.483</td>
</tr>
</tbody>
</table>

Statistical test applied: One-way ANOVA. *p-value < 0.05 is statistically significant. **Highly significant. *Statistically significant.

### Table II: Statistical difference for all parameters of control group A and experimental groups B and C on intergroup comparison.

<table>
<thead>
<tr>
<th>Parameters (µm)</th>
<th>Group A vs. B</th>
<th>Group A vs. C</th>
<th>Group B vs. C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distal luminal diameter</td>
<td>0.007**</td>
<td>0.129</td>
<td>0.394</td>
</tr>
<tr>
<td>Distal tubular diameter</td>
<td>0.157</td>
<td>0.166</td>
<td>0.999</td>
</tr>
<tr>
<td>Proximal luminal diameter</td>
<td>0.008**</td>
<td>0.230</td>
<td>0.269</td>
</tr>
<tr>
<td>Proximal tubular diameter</td>
<td>0.018*</td>
<td>0.039*</td>
<td>0.939</td>
</tr>
<tr>
<td>Transvertical diameter of renal corpuscle</td>
<td>0.506</td>
<td>0.597</td>
<td>0.988</td>
</tr>
</tbody>
</table>

Statistical test applied: Post Hoc Tukey test. *p-value < 0.05 is statistically significant. **Highly significant. *Statistically significant

Luminal diameter was measured from the apical surface of a cell on one side of lumen to apical surface of a cell on the other side. Then the mean of all measurements was taken and that value was the final reading of that parameter for a particular specimen. The transvertical diameter of the renal corpuscle is the diameter of the renal corpuscle taken in both the horizontal and vertical directions. To measure it, both the transverse and vertical diameters of a renal corpuscle were taken and their mean was the transvertical diameter of that specimen. Three readings per slide were taken and the mean was calculated. The mean of both slides of one specimen was the final reading of that specimen.

Analysis of data was performed using SPSS software (Statistical Package for the Social Sciences) version 22. The results were denoted by mean ± SD (standard deviation). For intergroup comparison, ANOVA (analysis of variance) was applied for quantitative variables which was followed by Post Hoc Tukey’s test. Statistically significant results were considered at a p-value equal to or less than 0.05.

### RESULTS

The proximal tubular and luminal diameter, distal tubular and luminal diameter and transvertical diameter of renal corpuscle were increased in experimental group B as compared to control group A. In experimental group C, all the parameters were decreased as compared to group B on administration of alpha-tocopherol and they became closer to the control group A. The p-value for proximal and distal tubular diameter was significant whereas for distal luminal diameter, it was highly significant as mentioned in Table I.

When the results of all three groups were compared with each other, the proximal tubular diameter was significant between groups A and B and groups A and C. The proximal luminal diameter and distal luminal diameter were highly significant between groups A and B only. All other values on the intergroup comparison were not significant as shown in Table II.

Figure 1 shows the histomicrographs of the renal corpuscle when seen at 40X magnification.

Figure 2 shows the histomicrographs of the renal corpuscle when seen at 40X magnification.

### DISCUSSION

In the current study, when the diameters of proximal and distal convoluted tubules and their lumen in group B were observed, there was vacuolization of cytoplasm of renal tubules. Most of the cell lining of renal tubules was distended with occlusion of renal tubular lumen. The study demonstrated that carboplatin causes an increase in oxidative stress, generation of free radicals and cytokine-mediated renal tubular injury. The direct effect of carboplatin is that it causes DNA damage, peroxidation of lipids, dysfunction of mitochondria and protein kinase and caspases activation. It binds with DNA and forms intrastrand cross-links and causes a conformational change in DNA structure, hence, affecting its replication.12
Dilatation and degeneration of renal tubules after administration of platinum containing drugs were also observed in certain studies in which oxidative stress and decreased antioxidant defence mechanisms were described to be the causative factors.\textsuperscript{13,14}

Group C showed decreased proximal and distal tubular and luminal diameter as compared to group B. The effects of vitamin E on nephrotoxicity were demonstrated in a study\textsuperscript{15} in which the tubular dilatation and degeneration significantly reduced with vitamin E co-administration.\textsuperscript{15} Another study used amikacin as a nephrotoxic agent and co-administered alpha-tocopherol.\textsuperscript{16} Alpha-tocopherol performs antioxidant function and directs free radicals to react with a hydrogen atom from its molecule and hence, breaks the chain of free radical reaction and they become unreactive. It also reduces the concentration of tumour necrosis factor-alpha (TNF-\(\alpha\)) and so, has an anti-inflammatory potential.

Regarding mean diameter of renal corpuscle, it was increased in group B as compared to group A. Similar results were observed in certain studies and it was explained that it is due to overexpression of TNF-\(\alpha\) and interleukin-6 (IL-6). Cytokine TNF-\(\alpha\) has a significant role in host immune and inflammatory response. IL-6 is secreted by fibroblasts, lymphocytes and macrophages. \textit{In vitro} studies have shown the increase in TNF-\(\alpha\) occurs after platinum chemotherapeutic drugs administration from renal tubular cells. Hence, due to oxidative stress, the level of cytokines is raised thereby resulting in kidney injury.\textsuperscript{14,17,18}

The reason for the altered histological size of renal corpuscle was explained by damage to the renal vasculature and vasoconstriction,\textsuperscript{19} which is due to dysfunctional endothelium. There is decreased glomerular filtration rate (GFR) along with decreased blood flow to kidneys, which is associated with increased renal vascular resistance. The mechanisms that regulate ultrafiltration depend on the contractile action of intraglomerular mesangial cells. Platinum compounds at high dose cause proliferation of mesangial cells. The contraction of mesangial cells causes a decrease in GFR by decreasing capillary surface area and permeability.

A decrease in diameter of renal corpuscle was seen in group C when compared with group B. The diameter was increased as compared to group A. The results were comparable to certain studies in which alpha-tocopherol administration showed a decrease in renal corpuscle diameter. The partial improvement in renal histological parameters is due to the strong antioxidant properties of alpha-tocopherol which reverses the oxidative damage caused to the renal cells by nephrotoxic agents.\textsuperscript{15,20,21} Similar renoprotective effect of alpha-tocopherol was observed in another study in which the severe renal toxicity of 90\% was reduced to only 6\% on its administration.\textsuperscript{22} Moreover, the antioxidant activity when combined with other antioxidants is potent enough to reverse the renal architecture to normal.\textsuperscript{23}

It was demonstrated in a study that alpha-tocopherol, in the presence of Acrylamide which is nephrotoxic, helps in preserving renal cortex architecture and apparently normal-shaped renal corpuscle with decreased glomerular congestion and intact Bowman’s capsule was observed on histology.\textsuperscript{24} It is because of its antioxidant function and it also helps to retain the normal-structure and function of mesangial cells on ultrasound. Hence, the architecture of renal corpuscle and GFR is improved.

The limitations of the study are that the facility of electron microscopy is not available and hence, the study does not explain the ultrastructural changes occurring in renal cortical cells due to the administration of the medicines used.

**CONCLUSION**

The parameters which were increased in group B after the administration of carboplatin showed improvement in group C on the administration of alpha-tocopherol. The values of microscopic parameters of group C measured by micrometry became closer to control group A. It was hence concluded that alpha-tocopherol plays a protective role against carboplatin-induced renal injury in rat renal cortex.

**DISCLOSURE:**

This article is from a thesis project.

**FUNDING:**

National University of Medical Sciences (NUMS), Rawalpindi, Pakistan.

**COMPETING INTEREST:**

The authors declared no competing interest.

**AUTHORS’ CONTRIBUTION:**

MA: Principal investigator, conception and design investigations of work, and write up.

KQ: Supervisor, critical analysis, and data analysis and interpretation.

FU, TF, AZ: Data analysis, SPSS analysis, and proof-reading.

AS: Histological slides making, gross examination, and handling of the specimen.

All the authors have approved the final version of the manuscript to be published.

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