

Amelioration of Carboplatin-Induced Changes in Rat Renal Cortical Tubules by Alpha-Tocopherol

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ABSTRACT

Objective: To determine the effects of carboplatin and alpha-tocopherol co-administration on gross kidney features and renal cortical tubules of carboplatin-treated rats.

Study Design: Laboratory-based experimental study.

Place and Duration of Study: Department of Anatomy, Army Medical College/National University of Medical Sciences Rawalpindi Pakistan, in collaboration with the National Institute of Health (NIH) and Pathology Lab Pak Emirates Rawalpindi Pakistan, from Jan to Dec, 2021.

Methodology: Thirty adults "Sprague-Dawley rats" of both genders were used and were divided into three Groups. Group-A was the Control Group. Groups B and C were given injection carboplatin 2.5 mg/kg body weight intraperitoneally on the first day. Group-C was given vitamin E at a dose of 62.7 mg/kg body weight daily via oral gavage starting from the second day of the experiment till the end of 12 weeks. Rats were euthanized at the end, kidneys were dissected, and they were preserved in 10% formalin after gross inspection. Tissue processing and staining were performed. A microscopic study was done to observe proximal and distal tubule necrosis.

Results: Thirty adult Sprague Dawley rats, of age 10-12 weeks, and weight 250.0±50.0 grams, were included, there was statistically significant difference in the animal weight difference ($p=0.001$), length of right kidneys (cm) ($p=0.002$), width of right kidney (cm) ($p=0.001$) and weight of right kidney (gm) ($p=0.012$) kidneys and relative tissue body weight index (RTBWI) ($p=0.048$) of Control Group-A and Experimental Group B and C.

Conclusion: Alpha-tocopherol has an ameliorative effect on gross features of rat kidneys and renal cortical tubular necrosis after carboplatin-induced renal damage.

Keywords: Alpha-tocopherol, Antioxidant, Carboplatin, Nephrotoxic, Renal cortex.

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INTRODUCTION

Carboplatin is a second-generation platinum-containing antitumor drug used to treat ovarian, head, neck, testicular and small cell lung cancers.¹ Carboplatin was developed to achieve improved clinical effects compared with cisplatin, its parent drug.² Carboplatin is used more as compared to cisplatin because of its aqueous solubility.³ and due to its decreased adverse effects. However, it is nephrotoxic, hepatotoxic and ototoxic.⁴ Due to its cytotoxicities, it is difficult for patients to tolerate it, and even if the adverse effects are present in minor form, the patient's quality of life is highly affected. Therefore, this drug must be given in a controlled dose to minimise its toxic effects.

Vitamin E is a group of lipid-soluble vitamins such as tocopherols and tocotrienols.⁵ Alphatocopherol is the most abundant form in nature and possesses antioxidant properties. The antioxidant function maintains the integrity of long-chain polyunsaturated fatty acids in cell membranes and is thus

necessary for their function. Vegetable oils are the major sources of alpha-tocopherol. When alphatocopherol is given as a dietary supplement within recommended levels, it improves the body's overall immunity.⁶ The stability of vitamin E depends on its form. Alpha-tocopherol is the most stable and abundant form in nature.⁷ It performs the functions of a radical peroxide scavenger that terminates chain reactions and protects from reactive oxygen species. In chemotherapy, reactive oxygen species are generated and decreased antioxidant levels along with chemotherapy induced toxicity.⁸

Carboplatin is a nephrotoxic chemotherapeutic drug as it is mainly excreted from the body through the renal pathway.⁹ It brings about kidney injury by vascular and cellular changes and alterations in molecular pathways.¹⁰ The individual effect of alphatocopherol on carboplatin-treated kidneys has not been studied. This study evaluated the histomorphological effect of co-administration of alpha-tocopherol with carboplatin on rat renal cortex. It will be evidence-based knowledge for oncologists to develop supportive therapy for cancer patients undergoing carboplatin chemotherapy.

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The study aimed to evaluate the effect of vitamin E co-administration with carboplatin on the histomorphology of rat renal cortex.

METHODOLOGY

This was a laboratory-based experiment conducted at the Department of Anatomy, Army Medical College/National University of Medical Sciences Rawalpindi, in collaboration with the National Institute of Health (NIH) and Pathology Lab Pak Emirates, Rawalpindi Pakistan from January to December 2021.

All animal care and handling procedures were performed after getting approval from the Ethics Committee of Army Medical College/National University of Medical Sciences, Rawalpindi Pakistan. (ERC/ID/128, dated 15 July, 2021). The Committee strictly followed the rules and regulations regarding the handling and care of animals.

Inclusion Criteria: Adult Sprague-Dawley rats of both genders, aged ten to twelve weeks and weighing 250 ± 50 grams were included in the study.

Exclusion Criteria: Apparently sick animals were excluded. Mice with any obvious injury and disease were excluded.

Thirty, Sprague-Dawley rats from the NIH, Islamabad animal house were included in the study. Male and female rats were housed separately. They were kept at a standard temperature of $21 \pm 2^{\circ}\text{C}$ in a room maintained on a 12-hour light/dark cycle and in standard humidity conditions. Rats were randomly divided into three Groups, each having ten rats. Each Group had five males and five females per Group who were kept in separate cages to avoid mating.

Group-A (Control Group): They were housed in standard conditions and were given a standard diet and water and Libitum for 12 weeks. **Group-B (Experimental Group):** The animals were housed in standard conditions and were administered a single intraperitoneal injection of carboplatin on the first day of the experiment in addition to a standard diet and water.

Group-C (Experimental Group): The animals were housed in standard conditions and were administered a single intraperitoneal injection of carboplatin on the first day of the experiment. They were given an oral dose of vitamin E daily via oral gavage from the second day of study till the end of 12 weeks, in addition to a standard diet and water.

All rats were weighed at the start and the end of the experiment by triple beam balance. In addition, the mean body weight of rats for each Group was taken.

Carboplatin was purchased as a 450mg injection from Korea United Pharm. Inc. in the name of "Carbotinol", a premixed aqueous solution of 10 mg/ml Carboplatin. The solution was diluted to a concentration of 0.5 mg/ml in 1000ml of 5% Dextrose water. The dose of carboplatin was 2.5 mg/kg in rats,¹¹ and was administered intraperitoneally.

Alpha-tocopherol was obtained from "Evion" capsules from Martin Dow Marker Ltd, each capsule having 200mg alpha-tocopherol. According to the study done by,¹² the dose of alpha-tocopherol given to rats was 62.7 mg/kg daily. However, it was administered to the animals of experimental Group-C only daily via oral gavage.

The rats were euthanized at the end of 12 weeks, kidneys were dissected, and both kidneys were weighed on the digital weighing scale. Right kidneys were washed with normal saline and were observed for gross study. They were examined by hand lens for colour. The texture was noted by palpation. Size (length and width) was measured using a Vernier calliper. Coronal sections were taken and examined for internal colour and appearance.

Relative tissue body weight index(RTBWI) was calculated using the following formula,¹³ $\text{RTBWI} = \frac{\text{Weight of organ in grams} \times 100}{\text{Weight of body in grams}}$. The tissues were then fixed and processed. Tissue blocks were made, and slides were prepared. One block per specimen and two slides per block were made. Staining was done with Haematoxylin & Eosin (H&E) stain.

Cortex of right kidney with proximal(PCT) and distal convoluted tubules(DCT) was observed under Olympus light microscope at 40X. Histological changes were assessed in the renal cortex in the form of the proximal and distal tubules necrosis.

In renal tubular necrosis, the tubules exhibit no nuclei, intensely eosinophilic homogenous cytoplasm, but the shape of the tubular cells is preserved. The necrotic cells fall into the tubular lumen and obstruct it. Cells which undergo necrosis show less or no basophilia and increased eosinophilia on H & E staining. The basement membrane is intact so that the tubular epithelium can regenerate.

The H & E stained slides were assessed for renal necrosis. Three random fields of the slide were selected; one field each from both poles of the kidney cortex and one from the mid portion. The severity of necrotic changes was graded according to the scale

used by,¹⁴ Level 0 (none): absence of necrosis, Level I (light necrosis): average of 1-2 tubules displaying necrosis in randomly selected three views of any slide by using a light microscope at 40X objective power, Level II (medium necrosis): average 3-5 tubules

The statistical difference for the animal weight difference, length, width and Weight of kidneys and RTBWI (relative tissue body weight index) in the intergroup comparison of Control Group A and Experimental Groups B and C was shown in Table-II.

Table-I: Mean Values of Animal Weight Difference, Length, Width and Weight of Kidneys and Relative Tissue Body Weight Index (RTBWI) of Control Group A and Experimental Group B and C (n=30)

Parameters	Group A Mean ± SD (n=10)	Group B Mean ± SD (n=10)	Group C Mean ± SD (n=10)	p-value
Animal weight difference (gm)	0.900 ± 1.197 weight gain	3.80 ± 1.032 weight loss	0.60±1.349 weight loss	0.001
Weight of right kidneys (gm)	2.17±0.290	1.89±0.159	1.88±0.209	0.012
Relative Tissue Body Weight Index	0.806±0.095	0.72±0.69	0.72±0.073	0.048
Length of right kidneys (cm)	2.24±0.164	1.97±0.182	1.96±0.195	0.002
Width of right kidneys (cm)	1.61±0.152	1.36±0.126	1.31±0.213	0.001

Table-II: Statistical difference for animal weight difference, length, width and weight of kidneys and RTBWI (relative tissue body weight index) on intergroup comparison of Control Group A and Experimental Groups B and C (n=30)

Parameters	Group A vs. B	Group A vs. C	Group B vs. C
Animal weight difference (gm)	0.001	0.001	0.001
Weight of right kidney (gm)	0.027	0.021	0.995
Relative Tissue Body Weight Index	0.079	0.079	1.000
Length of right kidneys (cm)	0.007	0.005	0.992
Width of right kidneys (cm)	0.007	0.001	0.785

displaying necrosis, Level III (severe necrosis): average of 6-10 tubules displaying necrosis, Level IV (extra severe necrosis): more than ten tubules displaying necrosis The mean of two slides and the final reading was the number of tubules displaying necrosis per specimen.

All data was analyzed using the software SPSS (Statistical Package for Social Sciences) version 22. The results were expressed as Mean±SD (Standard deviation). ANOVA (analysis of variance) was applied for intergroup comparison of quantitative variables followed by Post Hoc Tukey's test. Qualitative variables were presented as frequency and percentages. Finally, the Chi-square test followed by Fisher's exact test was applied to compare qualitative variables. The results were considered statistically significant at a *p*-value equal to or less than 0.05.

RESULTS

Thirty adult Sprague Dawley rats were included, aged 10-12 weeks, weighing 250.0±50.0 grams. There was statistically significant difference in the animal weight difference (*p*= 0.001), length of right kidneys (cm) (*p*= 0.002), the width of the right kidney (cm) (*p*= 0.001) and Weight of right kidney (gm) (*p*= 0.012) kidneys and relative tissue body weight index (RTBWI) (*p*= 0.048) of Control Group-A and experimental Groups B and C was shown in Table-I.

Right after dissection, the kidney specimens of all animals were observed. In control group A, kidneys appeared fresh and were reddish brown on the outside. In Experimental Groups B and C, the kidneys appeared pale and brown.

Regarding texture, in Control Group A, all specimens were soft. However, in Experimental Group B, the texture was firm. Finally, 60% of the specimen was soft in Experimental Group C, whereas 30% was firm.

Since the study is on the right kidney, the coronal section of the right kidney was taken. In Control Group A and Experimental Group C, the outer cortex was dark reddish brown, and the inner medulla was light reddish brown. In Experimental Group B, the outer cortex and inner medulla were dark reddish brown.

At 40X magnification, necrotic changes were observed in the renal cortex, as described in Figure.

DISCUSSION

In the current study, all the animals in control Group A and in experimental Group-B and C remained active with a normal sleep-wake cycle throughout the study period. The weight loss in Groups-B and C is in accordance with the study conducted by,¹¹ in which animals were given a maximum tolerated dose of platinum-containing compounds, and there was weight loss at the end of the study period. It was

because these compounds cause muscle atrophy and fatigue, thus leading to weight loss.

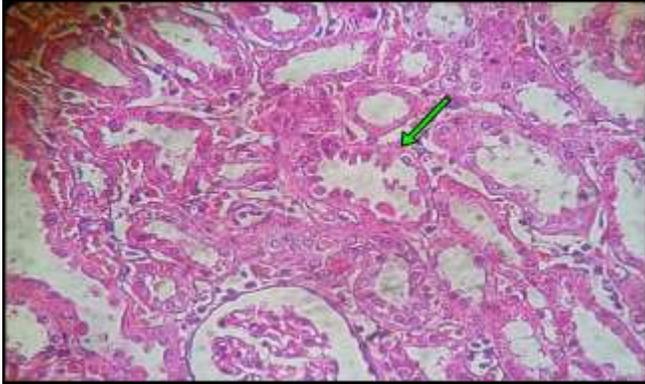


Figure: The green arrow points towards the necrotic tubule in photomicrograph of slide of animal B4. Picture taken at 40X magnification.

These two are one of the most common adverse effects of platinum compounds on skeletal muscle. Muscle wasting occurs because of the effects on muscle metabolism.^{12,13} Systemic inflammation occurs, which promotes muscle wasting by up-regulation of pro-inflammatory cytokines tumour necrosis factor-alpha (TNF- α) and interleukin-6(IL-6) and down-regulation of the anti-inflammatory cytokine interleukin-10(IL-10). Certain molecular mechanisms are involved in it, which include the Ubiquitin Proteasome Pathway,¹⁴ which causes protein degradation in skeletal muscle fibres and the Myostatin pathway, which results in decreased protein synthesis and decreased proliferation of myoblasts.^{15,16}

Group C showed a very less mean decrease in Weight, that is, 0.60mg. The improvement in Weight by co-administration of vitamin E is explained by a study in which breeder chickens were given vitamin E in varying quantities, resulting in weight gain due to enhanced immune response in terms of humoral immunity. It also results in decreased prostaglandin synthesis, thus resulting in an improved response to immunological stimuli.¹⁷

The size and Weight of kidneys of Groups B and C were reduced as compared to Group A. These findings were in accordance with the past study,¹⁸ which explained that the platinum compounds activate complex signalling pathways when the renal tubular cells are exposed to them which results in tubular injury. As a result, a highly potent inflammatory response initiates, thus resulting in damage to the kidney. This damage affects the renal parenchyma and renal

vasculature and circulation, leading to decreased blood flow and a decrease in glomerular filtration rate (GFR). Due to this decrease in perfusion, there is a decrease in the size of the kidneys.

In Group A, no necrosis (level 0) was seen. In Group B, severe necrosis (level III) was seen in 70% of the animals, whereas medium necrosis(level II) was in 30%. Statistical difference was significant when it was compared with Group A. These findings were comparable to other studies,¹⁹⁻²¹ which showed tubular degenerative changes, including desquamation and necrosis on histological sections. It was mentioned that oxidative stress plays an important role in causing the nephrotoxicity of carboplatin by generating free radicals and reactive oxygen species. Moreover, it results in depletion of renal antioxidant defence mechanisms resulting in cellular damage and necrosis.

The findings were comparable to the past literature,²² which showed that the administration of platinum compounds not only deranges the histological and biochemical parameters. These compounds accumulate in the lining epithelium of the proximal tubular cells due to a complex combination of apoptosis, oxidative stress, inflammation and fibrogenesis. There is overexpression of fibrogenic factors like TGF- β 1 and down expression of cell proliferation biomarkers, resulting in cellular necrosis.

Improvement was seen in Group C 50% of the tubules showed mild necrosis (level I), and 50% showed medium necrosis(level II). These findings were compared with another study,²³ in which coadministration of vitamin E and a nephrotoxic agent results in improved renal histology and biochemical parameters. Similar histological results were observed in another study,²⁰ as vitamin E neutralizes the reactive oxygen species and up-regulates antioxidant enzymes, improving the cellular architecture distorted by oxidative damage. Furthermore, Erdemli *et al.* suggested that vitamin E specifically eliminates the peroxidation products, thus acting as a powerful antioxidant and improving renal morphology.²⁴

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CONCLUSION

In the present study, all the gross and microscopic parameters were significantly deranged in Group B and improved in Group C. Therefore, it is concluded that alpha-tocopherol ameliorates the histomorphology of the renal cortex after carboplatin-induced renal damage.

Conflict of Interest: None.

Author's Contributions

MA: Principal investigator, KQ: Supervisor in research and write up, ZI: Data analysis and proof reading, SB: Data analysis and proof reading.

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