Iraqi Journal of Veterinary Sciences

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The effect of vitamin E on the histological structure of kidney in rats treated with cyclophosphamide

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Article information

Article history:
Received June 29, 2021
Accepted October 15, 2021
Available online March 13, 2022

Keywords:
Vitamin E
Cyclophosphamide
Kidney
Histological
Rats

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Abstract

Cyclophosphamide (CP) is a cytotoxic alkylating drug that is commonly used to treat autoimmune disorders and cancer. CP causes nephrotoxicity by producing highly reactive radicals devoid of oxygen. A fat-soluble vitamin E that interacts as a free radical scavenger, inhibiting nitrosamine production and blocking lipid peroxidation from polyunsaturated fatty acids. The aim of this research was to see if vitamin E may help combating CP induced nephrotoxicity. Thirty-two adult albino female Westar rats were randomly divided into four groups (n=8) and received daily treatment for thirty days. Group A considered as control group, receiving normal saline IP at dose of 0.3 ml, group B given 15 mg/kg b.w. of cyclophosphamide IP, while group C received cyclophosphamide with vitamin E at dose 200 mg/kg, oral administration of vitamin E one hour before injection of cyclophosphamide, in group D they received vitamin E orally at dose of 200 mg kg body weight mixed with olive oil daily by oral gavage. In group B the cyclophosphamide causes highly significant reduction in body weight and histologically showed atrophy of most glomeruli, distention in bowman's space, tubular degeneration and luminal hyaline cast material, while in group C showed most of the glomeruli normal except atrophy of few number of glomeruli and focal epithelial degeneration of renal tubules. Our study found that one of the primary mechanisms induced by cyclophosphamide may be nephrotoxicity due to oxidative stress, and pretreatment with vitamin E reduces cyclophosphamide-induced nephrotoxicity.

DOI: 10.33899/ijvs.2021.130689.1865,

Introduction

The kidneys are two reddish brown organs in the lumbar region of the body that are retroperitoneal on either side of the spinal column. Many homeostatic processes are controlled by the kidney. It regulates the amount of water, potassium, sodium chloride, phosphate, and a variety of other chemicals in the body to maintain the general chemical composition of the intracellular environment (1).

Cyclophosphamide (CP) is an antineoplastic drug that is used to treat cancers such lymphoma, leukemia, multiple myeloma, breast cancer, and autoimmune disorders (2).

Cyclophosphamide is converted into two harmful metabolites, acrolein and phosphoramide mustard, after being metabolically activated by liver enzymes cytochrome P450 (3). On enzymatic activation, these cytotoxic metabolites create covalent connections with DNA and proteins, resulting in cell death (4). Oxidative stress plays a role in CP causes nephrotoxicity (5).

The active metabolite of cyclophosphamide, acrolein, interferes with tissues' antioxidant defense mechanism and produces extremely reactive, oxygen-free radicals (6,7). During CP treatment, antioxidants should be given to detoxify the acrolein. As a result, a new drug is required to protect normal tissues from the toxicity of CP (8).

Vitamin E was discovered by Evans and Bishop in 1922 and it was isolated from wheat germ oil in 1936 (9). Vitamin E is a fat-soluble vitamin that acts as a free radical scavenger for blocking lipid peroxidation from polyunsaturated fatty acids and preventing nitrosamine formation (10). Vitamin E

is an essential biological system antioxidant that is abundant in tissues, particularly in membrane-rich sections (11). Administration of vitamin E can be used with other vitamins to support activities of antioxidant enzymes such catalase (12).

We aimed in current study to stud the cyclophosphamide effect on the histological structure of the kidney, and the protective effect provided by fat-soluble vitamin E on kidney injury caused by cyclophosphamide, in addition to the effect of the vitamin E on the histological structure of kidney.

Materials and methods

Animals

Thirty-two adult albino female Wister rats, measuring 200–250-gram body weight and three months old, were used in this study. They were obtained from the Experimental Research Unit, College of Veterinary Medicine, University of Mosul, Iraq.

The animals were given a week to adapt before the study begins, and they were kept in a quiet, temperature-controlled environment. They were given access to water and were fed daily. These animals were housed in sterilized plastic cages with homogenized wood shavings as bedding.

The experiments were carried out in compliance with internationally established ethical guidelines for the use and care of animals in laboratories.

Design of experiment

The animal of 32 female rates divided in to 4 group, each group of 8 rates. The control group A group administrated normal saline Intraperitoneally dose of 0.3 ml, group B which administrated dose of 15 mg/kg of cyclophosphamide intraperitoneally, group C given vitamin E orally dose of 200 mg/kg one hours before administrated cyclophosphamide 15 mg/kg Intraperitoneal, group D administrated vitamin E orally dose of 200 mg/kg, all group treated for 30 days.

Histopathological assessments

The female rates were anesthetized with ether. all of the kidneys were fixed in a 10% neutral buffered formalin solution. They were then dehydrated and embedded in paraffin in various alcohol concentrations. 4 µm sections were cut and stained with hematoxylin and eosin (H&E) (13). These sections were then examined for histopathological changes

Statistical analysis

The paired T-test was used for statistical analysis to find the changes in the body weight expressed by means and standard deviation (SD) before and after end of experiment in each group (Table 1).

Results

In group A rats intraperitonial injection of normal saline single daily dose for 30 consecutive dose. Rats in this group were normal, active, with good food intake, the difference in the body weight before and after the injection was found with no significant different in body weight (Table 1).

Histological structure of kidney showed normal, the bowman's space and glomeruli can be seen normally and renal tubules are normal (Figure 1: 1-3).

The rats in group B become less active, lethargic, poor feeding compared with the groups A, C, and E, this showed bleeding from some parts of the body. The difference in the body weight before and after injection was highly significant. Histologically showed atrophy of most glomeruli, with widening in bowman's space (Figure 1: 4 and 5).

The tubules showed degenerative change including cellular swelling and foamy cytoplasm (Figure 1: 6 and 7) also there is prominent changes in tubules includes swelling cells with eosinophilic nucleus appearance. some tubule showed luminal hyaline cast material (Figure 1: 8) there is interstitial bleeding with inflammatory cell infiltration (Figure 1: 9).

In group C the rats were active and food intake was normal. The difference in the body weight before and after injection was significant. Histologically most of the glomeruli in the section looked normal except few number of glomeruli where atrophy with bowman's space widening (Figure 1: 10).

Renal tubules. Showed focal epithelial degeneration, change with patent lumen in most of tubule as well as absent of hyaline cast (Figure 1: 11).

In group E the rats showed active with good food intake and no significant deference in the body weight before and after injection was not significant. Examination of the kidney sections showed normal histological features including normal glomeruli and renal tubule normal with absence of degenerative changes (Figure 1: 12).

Table 1: Body weight changes in each group of rats before and after injection

Group	Body weight (gm)		
	Before injection	After injection	P Values
A	226.125±12.218	235.375±10.528	0.12700
B	218.250±17.119	191.250±13.231	0.00333
C	215.250±19.137	186.125±19.802	0.00972
D	208.625±16.422	221.375±12.023	0.04910

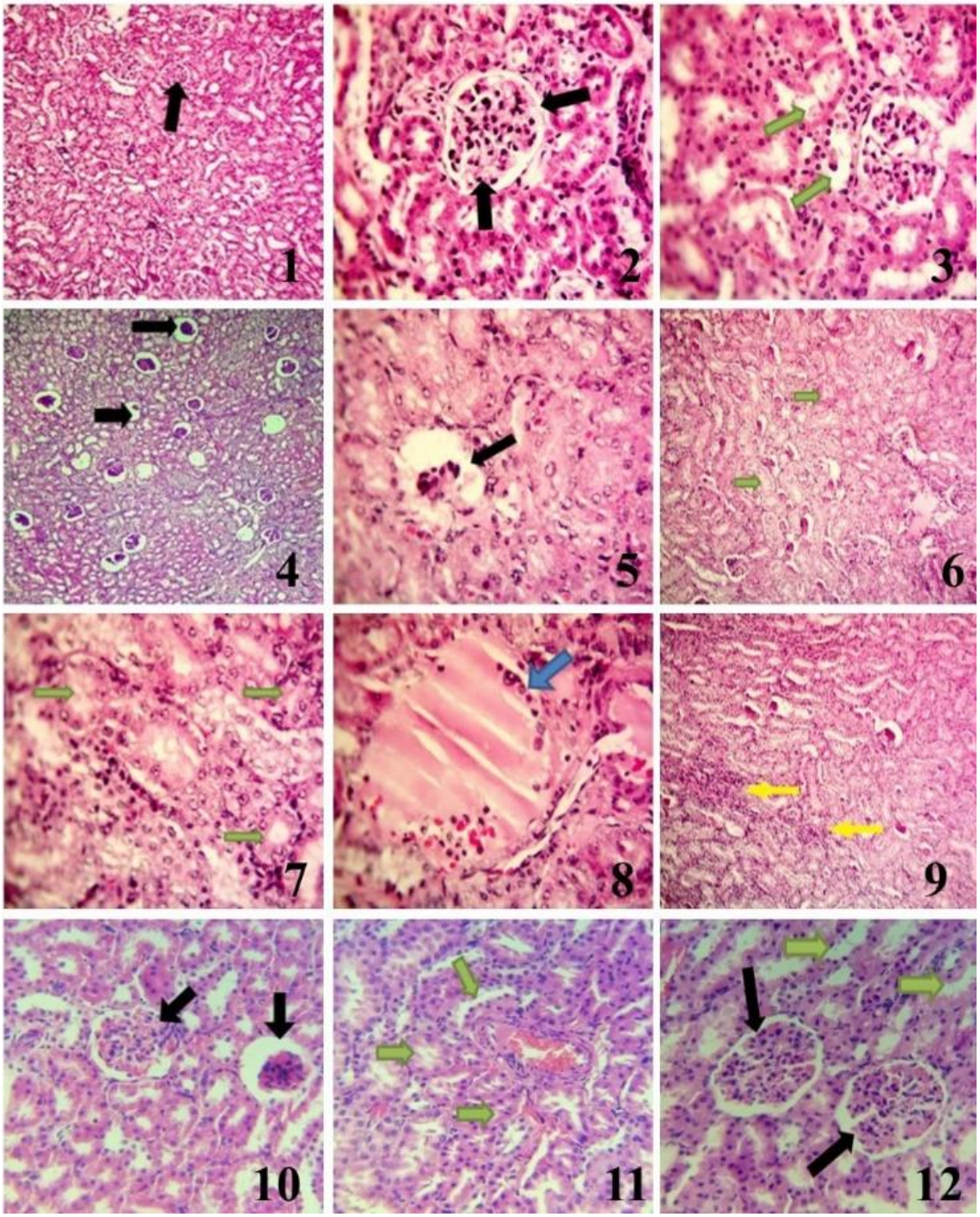

Figure 1: Sections in kidney. (1) control group, group A; normal glomeruli and normal bowman space (black arrow). (2) control group, group A; normal glomeruli and normal bowman space (black arrow). (3) group A; normal tubules with basally located nuclei (green arrow). (4) group B; glomeruli with collapse, atrophy and widening of bowman's space (black arrow). (5) group B; glomeruli with collapse, atrophy and widening of bowman's space (black arrow). (6) group B; degenerative change including cellular swelling, foamy cytoplasm (green arrows). (7) Group B; degenerative change including cellular swelling, foamy cytoplasm (green arrows). (8) group B; tubule luminal hyaline cast material (blue arrow). (9) group B; interstitial inflammatory infiltration (yellow arrow). (10) group C; improvement in histological structure (black arrow). (11) group C; improvement in histological structure (green arrow). (12) group D; normal glomeruli and normal bowman space (black arrow), normal tubules (green arrow).

Discussion

The kidneys are vital metabolic organs that filter urine and eliminate metabolic waste and chemical compounds, as well as maintain homeostasis and proper physiological functions (14). Chemotherapy improves the quality of life for cancer patients. On the other hand, it has a variety of harmful effects on normal cells and tissue (15). Regarding rats treated with cyclophosphamide causing loss of activity and appetite. The difference in body weight before and after cyclophosphamide treatment could be related to decreased adipose tissue and proteins, which could be related to anti-cancer drugs causing appetite loss. Increase protein catabolism and decreasing food consumption utilization with Excessive loss of water, salts, and proteins as a result of kidney injury, dehydration, and weight loss in anti-cancer drug-treated rats due to gastrointestinal toxicity and subsequent decrease in food intake with loss of appetite for the animal or due to excessive loss of water, salts, and proteins as a result of kidney injury, dehydration, and weight loss (16,17). Previous research has found that cyclophosphamide causes glomerular congestion and degeneration in Bowman's space and collective tubules of the kidneys, Glomeruli atrophy, Bowman's capsular spaces are considerably increased, and the parietal and visceral layers of the kidney are affected (18,4). Because its active metabolites create reactive oxygen species, cyclophosphamide has been found to promote kidney alteration and oxidative stress-induced renal injury (19,20). As a result of cell injury and distraction of cytoplasmic organelle such as lysosome, the activity of lysosome enzymes were observed to be decreased in the kidneys of rats given CP (21). Because of the lysosomes serve as the cell's digestive system, it serves as proteolytic enzymes for protein hydrolysis. Dysfunction of lysosomes can result in a reduction in protein digestion, as lysosomes degrade proteins on a regular basis. Protein half-lives are lengthened as a result. This causes an abnormally large amount of proteins to accumulate within the cell. Protein content has increased Although an increase in the ratio of protein to DNA is regarded a better evidence of hypertrophy, it can be employed alone as an indicator (21). Cyclophosphamide caused glomerulonephritis, cortical tubular vacuolization and necrosis, acidophilic material accumulation in tubules, and interstitial edema and hemorrhage in kidney of the rat's (5,22). Cyclophosphamide causes tubular necrosis and desquamation of the lining epithelial cells of the kidney tubules, resulting in the buildup of acidic materials within the tubular lumen. Inflammatory cell infiltration, tubular edema, and cortical tubular degeneration are all symptoms of tubular degeneration (23). Vitamin E biological chain breaking antioxidant that protects cells and tissues from free radicals (24). Decrease renal injuries caused by cyclophosphamide-induced-nephrotoxicity due to antioxidant substance vitamin E (16).

Conclusions

Giving cyclophosphamide for 30 day reduces body weight and affects the histological stricture of the kidney which can be ameliorated by the use of vitamin E and reduces this change

Acknowledgment

We would like to thanks to all those who provided me the possibility to finished this article, specially staff of animal house in Mosul University's College of Veterinary Medicine.

Conflict of interest

According to the authors, there is no conflict of interest.

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تأثير فيتامين هـ على التركيب النسيجي للكلية في الجرذان المعالجة بالسيكلوفوسفاميد

احمد علاوي عبيد، مها عبد الجبار السماك ومصطفى صلاح فاضل

فرع التشريح، كلية الطب، جامعة الموصل، الموصل، العراق

الخلاصة

السيكلوفوسفاميد هو دواء ذو تأثير سام على الخلايا يستخدم بشكل شائع لعلاج الأمراض السرطانية واضطرابات المناعة الذاتية. يسبب السيكلوفوسفاميد التسمم الكلوي من خلال إنتاج جذور حرة شديدة التفاعل. يعتبر فيتامين هـ من الفيتامينات الذائبة في الدهون ويعمل كعامل مؤكسد مما يساعد على إزالة الجذور الحرة، يثبط إنتاج النتر وزامين ويمنع تكون بيروكسيد الدهون من الأحماض الدهنية المتعددة الغير مشبعة. كان الهدف من هذه الدراسة هو معرفة ما إذا كان فيتامين هـ يعمل على تقليل أو إزالة التسمم الكلوي الحاصل نتيجة إعطاء السيكلوفوسفاميد. تم تقسيم اثنان وثلاثين أنثى بالغة من الجرذان البيضاء إلى أربعة مجاميع (ن=٨) وتلقت علاجا يوميا لمدة ثلاثون يوما. المجموعة أ: مجموعة السيطرة، إعطاء جرعة محددة من المحلول الملحي حقنا داخل الصفاق بجرعة ٠,٣ مل. المجموعة ب: تم إعطائها جرعة محددة من السيكلوفوسفاميد حقنا داخل الصفاق بجرعة ١٥ ملجم/جم من وزن الجسم. أما المجموعة ج: فتم إعطائها فيتامين هـ بجرعة ٢٠٠ ملجم/كجم عن طريق الفم قبل ساعة من إعطاء الجرذان السيكلوفوسفاميد بجرعة ١٥ ملجم/كجم من وزن الجسم حقنا داخل الصفاق. أما المجموعة د: فتم إعطائها فيتامين هـ عن طريق الفم بجرعة ٢٠٠ ملجم/ كجم من وزن الجسم. تسبب السيكلوفوسفاميد في المجموعة ب في انخفاض كبير وملحوظ في وزن الجسم واظهر نسيجيا ضمور لمعظم الكبيبات الكلوية وتوسع في أغشية بومان المغلفة للكبيبات الكلوية وتنخر للنبيبات الكلوية مع تجمع للمواد البروتينية داخل النبيبات الكلوية. أوجدت دراستنا أن السيكلوفوسفاميد يسبب التسمم الكلوي نتيجة الإجهاد التأكسدي، وان إعطاء فيتامين هـ يقلل التأثيرات السمية الناتجة عن السيكلوفوسفاميد.

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