Original Article

The Possible Protective Role of Vitamin E Against Deferasirox-Induced Injury of Renal Cortical Tubules in Adult Male Albino Rat: A Histological and Immunohistochemical Study

Shimaa M. Badr, Heba E. M. Sharaf Eldin and Marwa A. A. Ibrahim

Department of Histology and Cell Biology, Faculty of Medicine, Tanta University, Egypt

ABSTRACT

Introduction: Deferasirox, as an oral iron chelator, is presently the first-choice medication for iron overload caused by repeated blood transfusions due to its ease of use, efficacy, and high bioavailability, yet deferasirox has been also reported with the occurrence of acute kidney injury and tubular dysfunction. Vitamin E is a potent antioxidant and anti-inflammatory agent.
Aim of the Work: To study the effect of deferasirox on the oxidative status, histological structure, apoptosis, proliferation, and inflammation of the renal cortical tubules and examine the potential protective role of vitamin E against such effect in adult male albino rat.
Material and Methods: Twenty-four adult male albino rats were subdivided into four equal groups; control, vitamin E-treated (100 mg/kg/day vitamin E orally for 4weeks), deferasirox-treated group (100 mg/kg/day deferasirox orally for 4weeks), and vitamin E&deferasirox-treated group (concomitantly administered vitamin E and deferasirox). Kidney specimens were processed for different biochemical, histological, and immunohistochemical studies.
Results: Deferasirox-treated group revealed loss of the normal histological architecture of the renal cortex involving numerous nuclear and cytoplasmic alterations of renal cortical tubules with inflammatory signs. Tissue malonaldehyde level was significantly surged. A significant increase in caspase-3, Ki67, and iNOS immunohistochemical expression was recorded, whereas a significant drop in the histochemical expression of PAS was detected. Results from the group concomitantly administered with vitamin E and deferasirox exhibited an apparently normal histology of the renal cortex with a non-significant difference in all studied parameters compared to the control group.
Conclusion: Deferasirox administration led to histological alterations in the renal cortical tubules through inducing oxidative stress, apoptosis, proliferation, and inflammation. Concomitant supplementation with vitamin E exerted a protective action against deferasirox harmful effects most probably through its antioxidative, antiapoptotic, and anti-inflammatory properties.

Received: 06 June 2022, **Accepted:** 12 August 2022

Key Words: Deferasirox; immunohistochemistry; renal cortex; vitamin E.

Corresponding Author: Marwa A. A. Ibrahim, MD, Department of Histology and Cell Biology, Faculty of Medicine, Tanta University, Egypt, **Tel.**: +20 10 9918 6399, **E-mail:** marwa.ibrahim@med.tanta.edu.eg

ISSN: 1110-0559, Vol. 46, No. 4

INTRODUCTION

Chronic iron overload secondary to repeated blood transfusions affects almost all patients of sickle cell anemia, thalassemia, and myelodysplastic syndromes, as the human body does not have an efficient way for excess iron elimination. Iron overload usually causes multiple organs injury and dysfunction such as heart, liver, skin, endocrine glands, and joints. Therefore, iron chelation is essential to avoid organ dysfunction and decrease mortality[1,2,3].

Both parenteral and oral iron chelation decrease iron burden and organ injury. Deferasirox as an oral chelator is presently the first-choice medication for iron overload caused by repeated blood transfusions due to its ease of use, efficacy, and high bioavailability[3,4]. Gastrointestinal problems and increased liver enzymes are the most frequent adverse effects of deferasirox. One of the main side effects in about one third of patients treated with deferasirox is the rise of the level of serum creatinine[5,6]. In human, deferasirox has been also reported with the occurrence of acute kidney injury, Fanconi syndrome, and tubular dysfunction. Deferasirox kidney tubular damage presents with characteristic biochemical results including metabolic acidosis, hypophosphatemia, hypokalemia, phosphaturia, glucosuria, and aminoaciduria. The pattern of deferasirox-induced renal tubular damage is still inadequately cleared[7].

Vitamin E is a fat-soluble antioxidant naturally found in wheat germ, vegetable oils, and particular types of nuts and grains[8]. Vitamin E has an essential role in keeping tissues from extreme lipid peroxidation and preventing the propagation of free radicals[9]. It also has a great role in cell membrane function, elasticity, and integrity. Vitamin E is suggested as a preventive agent for kidney injuries accompanied with reactive oxygen species (ROS), such as occur with acute kidney injury induced by ischemia or exposure to nephrotoxic drugs[10,11,12].

Therefore, this study was planned to study the effect of deferasirox on the oxidative status, histological structure, apoptosis, proliferation, and inflammation of the renal cortical tubules and examine the potential protective role of vitamin E against such effect in adult male albino rat.

 DOI: 10.21608/ejh.2022.143261.1699

MATERIALS AND METHODS

Experimental design

Twenty-four adult male albino rats, each weighing 200-220g, were kept in clean appropriately aired cages under standard housing conditions at the animal house of the Histology department of Tanta Faculty of Medicine. They had access to balanced laboratory diet and water ad libitum. This study was permitted by the Research Ethics Committee of Tanta Faculty of Medicine (approval code #35531). The rats were allocated into four equal study groups:

Group I (Control group): the rats were further subdivided into two equal subgroups; Subgroup Ia; animals were kept without any treatment. Subgroup Ib; animals were orally gavaged with 1ml of corn oil daily along with 1ml of distilled water for 4weeks.

Group II (Vitamin E-treated group): the rats were orally gavaged with 100 mg/kg/day of vitamin E (95.5%, α-tocopherol) in 1ml of corn oil for 4weeks[13]. Vitamin E was purchased from Merck (cat # 258024, Darmstadt, Germany).

Group III (Deferasirox-treated group): the rats were orally gavaged with 100 mg/kg/day of deferasirox dissolved in 1ml of distilled water for 4weeks[14]. Deferasirox (Exjade® 500mg tablets) was purchased from Novartis (CAS 201530-41-8, Egypt).

Group IV (Vitamin E&deferasirox-treated group): the rats were concurrently administered vitamin E and deferasirox as explained in groups II and III respectively.

After the completion of the experiment, the rats were euthanized with pentobarbital (40mg/kg)[15]. The kidneys were rapidly dissected for biochemical study and light microscopy preparation.

Biochemical analysis

Spectrophotometric determination of the level of tissue malondialdehyde (MDA), as a marker of oxidative stress, was performed[16].

Light microscopy examination

Kidney specimens were fixed in 10% formalin and prepared for embedding in paraffin. Hematoxylin and eosin (H&E)[17] and Periodic Acid Schiff reagent (PAS)[18] staining was performed.

Immunohistochemical staining

The primary antibodies; rabbit polyclonal antibodies against cleaved caspase-3 (as an apoptosis marker), Ki67 (as a proliferation maker), and inducible nitric oxide synthase [iNOS] (as inflammation marker) (ab2302, ab15580, and ab3523 respectively, Abcam, USA) were used at dilutions of 1:200, 1:500, and 1:100 respectively. Kidney sections were processed according the standard Labeled Strept(Avidin) Biotin (LSAB) method as previously described[19], using 3,3′-diaminobenzidine (DAB) hydrogen peroxide and counterstaining with hematoxylin.

Morphometric analysis

Microphotograph acquisition was done employing a light microscope (Leica, Switzerland) fixed to a digital camera (Leica, Switzerland). Image analysis was done using "ImageJ" software (1.48v NIH, USA) was performed. Ten different fields from each section were quantified at x400 to estimate the mean color intensity of PAS, caspase-3, Ki67, and iNOS expression in addition to the mean percentage and area percentage of Ki67 and iNOS respectively.

Statistical analysis

One-way analysis of variance (ANOVA) was followed by Tukey's test for data analysis (IBM SPSS, IBM Corp, USA). If the probability value (p) was ≤ 0.05, the differences were recorded as significant.

RESULTS

Biochemical findings

Tissue malonaldehyde levels from deferasirox-treated group III was significantly higher ($p<0.001$) than the control group I, whereas its level in vitamin E&deferasirox-treated group IV was not significantly different ($p=0.0810$) from the control (Table 1).

H&E findings

Sections from both control group I and vitamin E-treated group II similarly exhibited the normal histological structure of renal cortex with the characteristic renal corpuscles and tubules. Renal corpuscles were composed of glomeruli of cluster of capillaries covered with the visceral layer of the Bowman's capsule and separated from its flat squamous parietal layer with a capsular space. The proximal convoluted tubules (PCT) were lined by cuboidal cells with rounded basal nuclei surrounding a narrow lumen. The distal convoluted tubules (DCT) were lined by cuboidal cells with spherical nuclei surrounding a wide lumen. Peritubular capillaries were observed in between the tubules (Figure 1).

Sections from deferasirox-treated group III revealed loss of the normal histological architecture of renal cortex. Congested, degenerated, and collapsed glomeruli with focal adhesions in the Bowman's capsule were detected. More importantly, most PCT were distorted with vacuolated cytoplasm and pyknotic nuclei. Many DCT were dilated with exfoliated epithelial cells or nuclei shed into the lumen. Some PCT and DCT were totally distorted and desquamated. Luminal acidophilic casts were occasionally observed in some DCT (Figures 2,3). Some extremely dilated congested blood vessels in addition to some areas of interstitial hemorrhage together with extensive mononuclear cellular infiltration were observed (Figure 4).

Sections from vitamin E&deferasirox-treated group IV depicted an apparently normal renal cortex histoarchitecture with glomeruli enclosed within intact Bowman's capsule with uniform capsular space. Most PCT and DCT were intact (Figure 5).

Periodic Acid Schiff (PAS) histochemical findings

Sections from both control group I and vitamin E-treated group II similarly depicted a strong PAS-positive expression in the intact brush border and regular basement membrane of PCT and DCT as well as the parietal layer of the Bowman's capsule appeared as magenta red color (Figure 6). While deferasirox-treated group III revealed a weak PAS-positive expression in the disrupted brush border and a weak to moderate reaction in the irregular basement membrane of many PCT and DCT. A moderate PAS expression with focal thickening of the parietal layer of the Bowman's capsule was observed (Figure 7). While sections from vitamin E&deferasirox-treated group IV showed a moderate to strong PAS-positive reaction in the brush border and a strong reaction in the basement membrane of PCT and DCT as well as the parietal layer of the Bowman's capsule (Figure 8).

The mean color intensity of PAS histochemical staining in group III demonstrated a significant decrease (p<0.001) compared to the control group, while group IV was not significantly different (p=0.0807) from the control (Table 1, Histogram 1-A).

Caspase-3 immunohistochemical findings

Immunohistochemically stained sections for detection of caspase-3 in both control group I and vitamin E-treated group II similarly showed a weak positive caspase-3 immunoreaction in the renal cortical tubules and corpuscles (Figure 9). While sections from deferasirox-treated group III showed a strong nuclear and/or cytoplasmic caspase-3 positive immunoreaction in the renal cortical tubules and corpuscles appeared as a brownish color (Figure 10). In contrast, vitamin E&deferasirox-treated group IV depicted a moderate caspase-3 immunoreaction particularly in the PCT and DCT (Figure 11).

The mean color intensity of caspase-3 immunoreaction from group III revealed a significant upregulation (p<0.001) with regard to the control group, whereas group IV reported a non-significant difference (p=0.0797) from the control (Table 1, Histogram 1-B).

Ki67 immunohistochemical findings

Immunohistochemically stained sections for detection of ki67 in both control group I and vitamin E-treated similarly revealed some Ki67-positive cells with a moderate immunoreaction in the renal cortical tubules and corpuscles appeared as a nuclear brownish color (Figure 12). While deferasirox-treated group III exhibited numerous Ki67-positive cells with a strong immunoreaction in the renal cortical tubules and corpuscles (Figure 13). Whereas sections from vitamin E&deferasirox-treated group IV depicted many Ki67-positive cells with a moderate to strong immunoreaction in most renal cortical tubules (Figure 14).

The mean percentage and color intensity of Ki67-positive cells from group III revealed a significant increase (p<0.001) with regard to the control group, whereas group IV recorded a non-significant difference (p=0.0817 and 0.1615 respectively) from the control (Table 1, Histogram 1-C).

iNOS immunohistochemical findings

Immunohistochemically stained sections for detection of iNOS in both control group I and vitamin E-treated similarly exhibited a faint positive iNOS cytoplasmic immunoreaction in few glomerular and interstitial cells exhibited as a brownish color (Figure 15). While deferasirox-treated group III revealed a strong iNOS cytoplasmic immunoreaction in numerous glomerular cells, peritubular capillaries, and interstitial cells (Figure 16). Whereas vitamin E&deferasirox-treated group IV showed a moderate cytoplasmic iNOS immunoreaction in some glomerular and interstitial cells (Figure 17).

Both mean color intensity and area percentage of iNOS positive expression from group III was significantly higher (p<0.001) than the control group, whereas group IV displayed a non- significant difference (p=0.1364 and 0.1202 respectively) compared to the control (Table 1, Histogram 1-D).

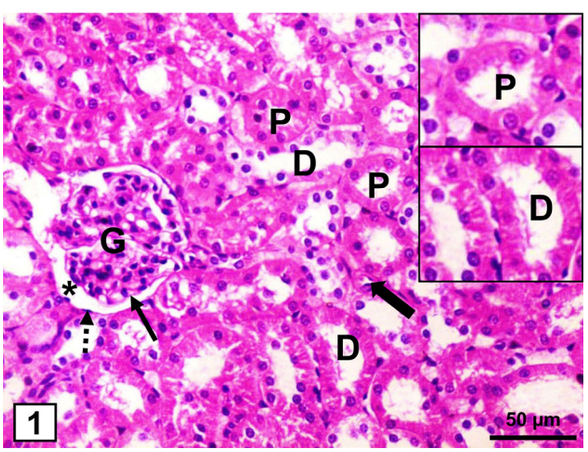


Fig. 1: Control group shows the normal histoarchitecture of renal cortex. Renal corpuscles are composed of glomeruli (G) of tuft of capillaries covered with the visceral layer (thin arrow) of the Bowman's capsule and separated from its flat squamous parietal layer (dashed thin arrow) with a capsular space (asterisk). The proximal convoluted tubules (P) are lined by cuboidal cells with rounded basal nuclei surrounding a narrow lumen. The distal convoluted tubules (D) are lined by cuboidal cells with spherical nuclei surrounding a wide lumen. Peritubular capillaries (thick arrow) are observed in between the tubules. [H&E x400, scale bar=50µm, insets x1000]

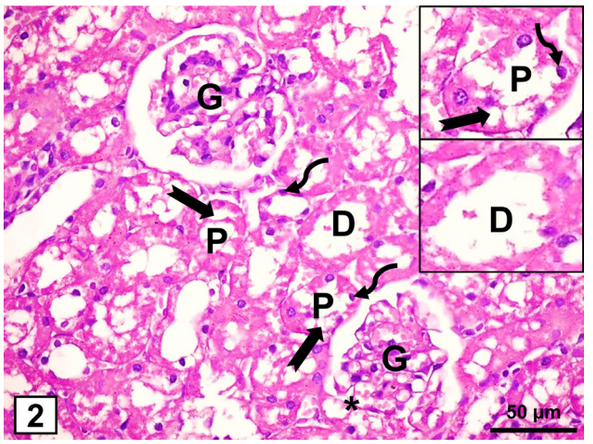


Fig. 2: Group III shows congested glomeruli (G) with focal adhesion (asterisk). Most proximal convoluted tubules (P) are distorted with vacuolated cytoplasm (notched arrows) and pyknotic nuclei (wavy arrows). Many distal convoluted tubules (D) are dilated showing exfoliated epithelial cells or nuclei shed into the lumen. Some tubules are totally distorted. [H&E x400, scale bar=50µm, insets x1000]

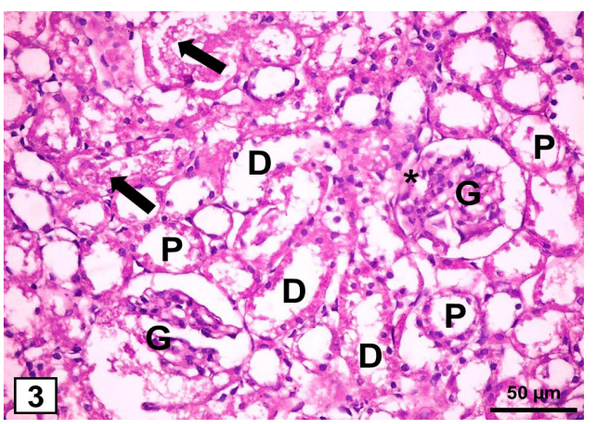


Fig. 3: Group III shows degenerated and collapsed glomeruli (G) with focal adhesion (asterisk). Most proximal convoluted tubules (P) and distal convoluted tubules (D) are extremely dilated and desquamated. Luminal acidophilic casts (thick arrows) are observed in some distal convoluted tubules. [H&E x400, scale bar=50µm]

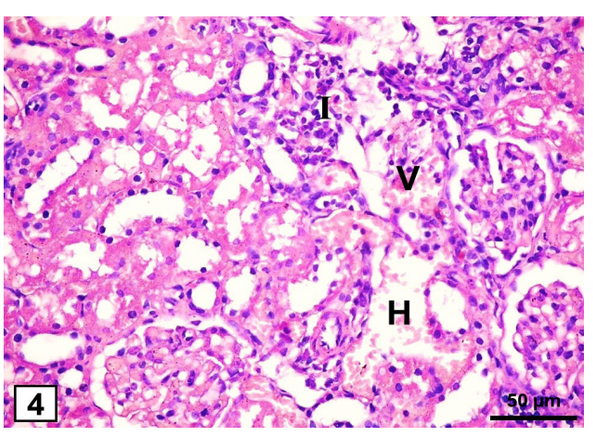


Fig. 4: Group III shows Some extremely dilated congested blood vessels (V) in addition to some areas of interstitial hemorrhage (H) together with extensive mononuclear cellular infiltration (I) are observed. [H&E x400, scale bar=50µm]

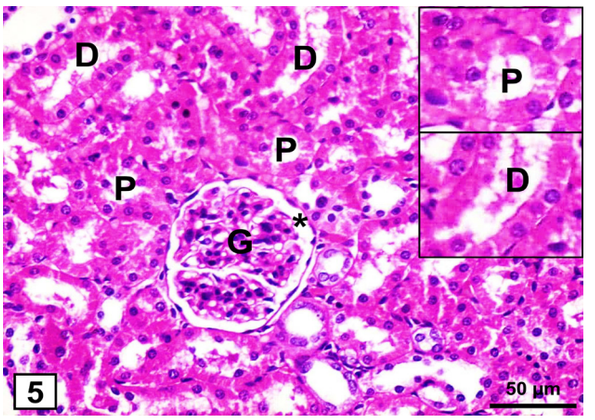


Fig. 5: Group IV shows a near control renal cortex histoarchitecture with glomeruli (G) enclosed within intact Bowman's capsule with uniform capsular space (asterisk). Most proximal convoluted tubules (P) and distal convoluted tubules (D) are intact. [H&E x400, scale bar=50µm, insets x1000]

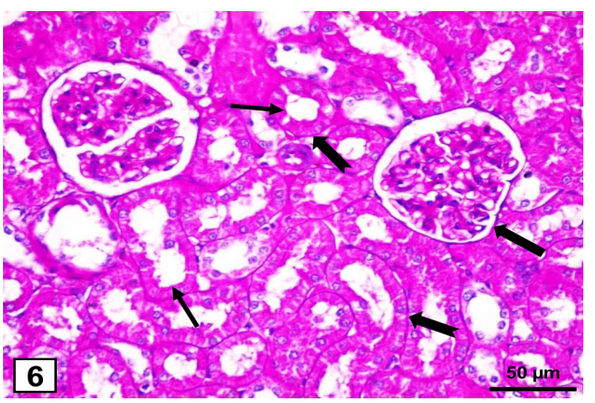


Fig. 6: Control group shows a strong PAS expression appears as a magenta red color in the intact brush border (thin arrows) and regular basement membrane (notched arrows) of proximal and distal convoluted tubules, and the parietal layer of the Bowman's capsule (thick arrow). [PAS x400, scale bar=50µm]

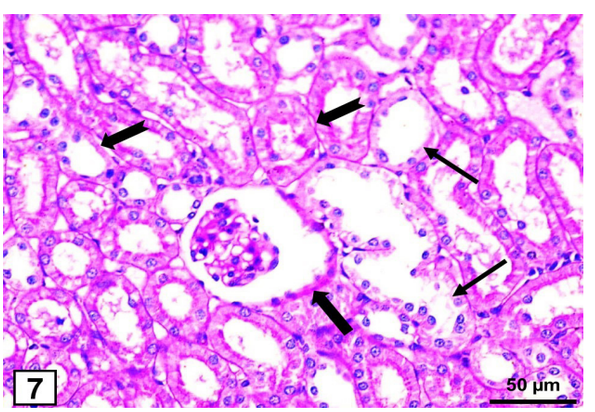


Fig. 7: Group III shows a weak PAS expression in the disrupted brush border (thin arrows) and a weak to moderate expression in the irregular basement membrane (notched arrow) of many proximal and distal convoluted tubules. A moderate PAS expression with focal thickening of the parietal layer of the Bowman's capsule (thick arrow) is observed. [PAS x400, scale bar=50µm]

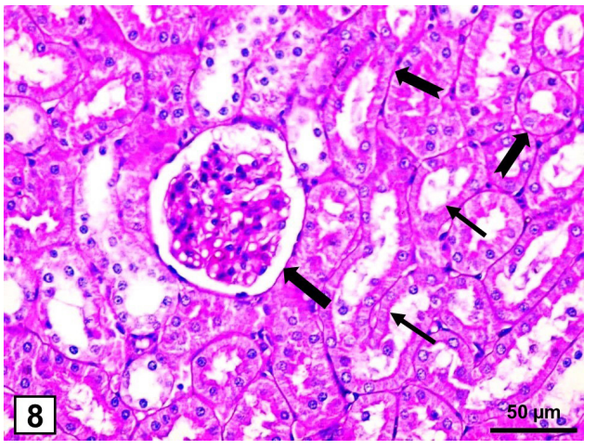


Fig. 8: Group IV shows a moderate to strong PAS expression in the brush border (thin arrows) and a strong expression in the basement membrane (notched arrow) of proximal and distal convoluted tubules, and the parietal layer of the Bowman's capsule (thick arrow). [PAS x400, scale bar=50µm]

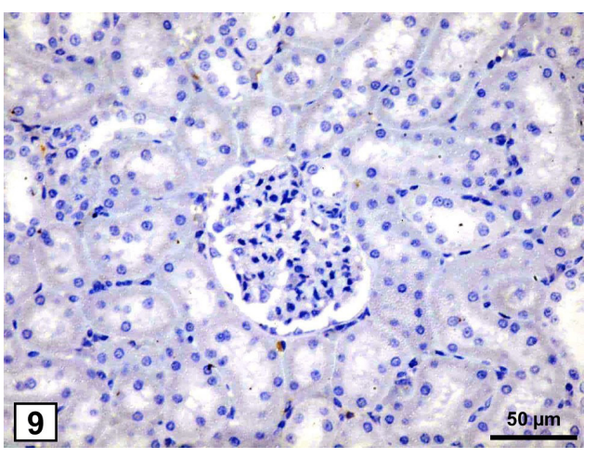


Fig. 9: Control group shows a weak positive caspase-3 immunoreaction in the renal cortical tubules (thick arrow) and corpuscles (thin arrow). [Caspase-3 x400, scale bar=50µm]

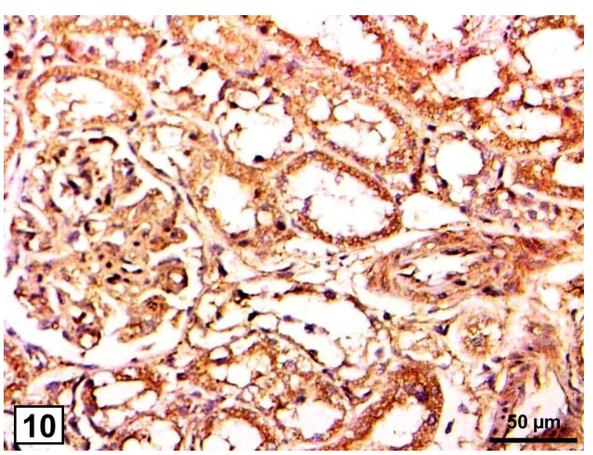


Fig. 10: Group III shows a strong positive nuclear and/or cytoplasmic caspase-3 immunoreaction in the renal cortical tubules (thick arrows) and corpuscles (thin arrows) appears as a brownish color. [Caspase-3 x400, scale bar=50µm]

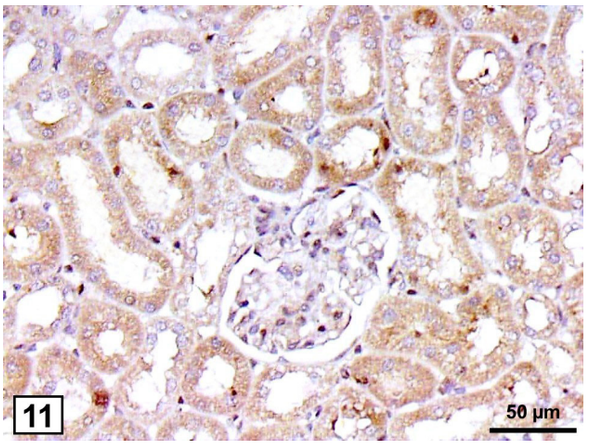


Fig. 11: Group IV shows a moderate caspase-3 immunoreaction particularly in the proximal and distal convoluted tubules (thick arrows). [Caspase-3 x400, scale bar=50µm]

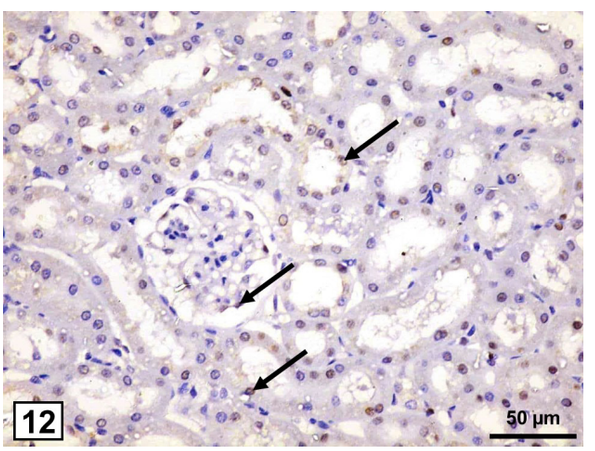


Fig. 12: Control group shows some Ki67-positive cells with a moderate immunoreaction in the renal cortical tubules (thick arrows) and corpuscles (thin arrow) as a nuclear brownish color. [Ki67 x400, scale bar=50µm]

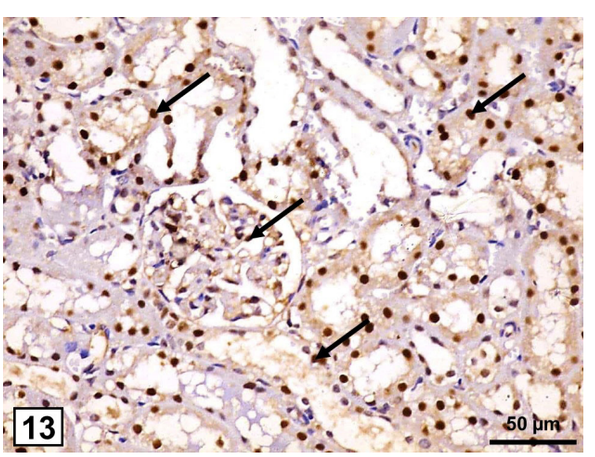


Fig. 13: Group III shows numerous Ki67-positive cells with a strong immunoreaction in the renal cortical tubules (thick arrows) and corpuscles (thin arrow). [Ki67 x400, scale bar=50µm]

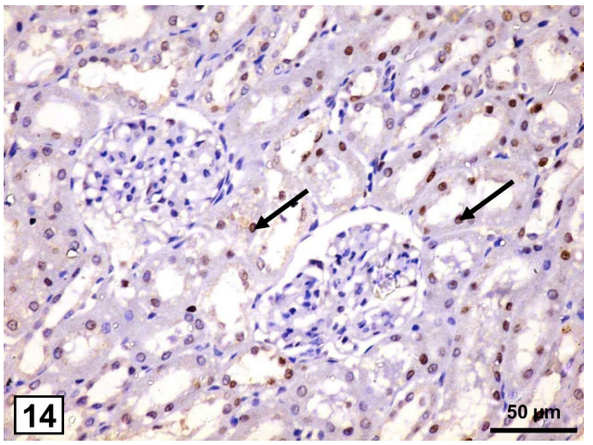


Fig. 14: Group IV shows many Ki67-positive cells with a moderate to strong immunoreaction in most renal cortical tubules (thick arrows). [Ki67 x400, scale bar=50µm]

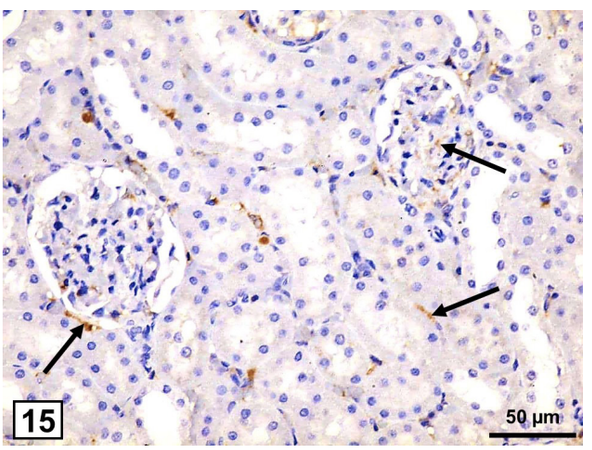


Fig. 15: Control group shows a faint positive iNOS cytoplasmic immunoreaction in few glomerular (thin arrows) and interstitial cells (thick arrow) appears as a brownish color. [iNOS x400, scale bar=50µm]

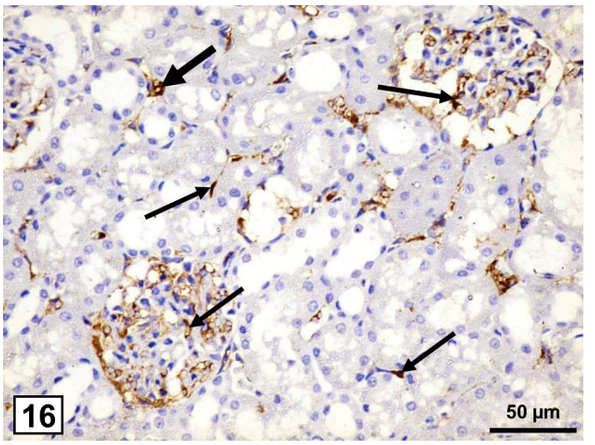


Fig. 16: Group III shows a strong positive cytoplasmic iNOS immunoreaction in numerous glomerular cells (thin arrows), interstitial cells (thick arrows), and peritubular capillaries (dashed arrow) [iNOS x400, scale bar=50µm]

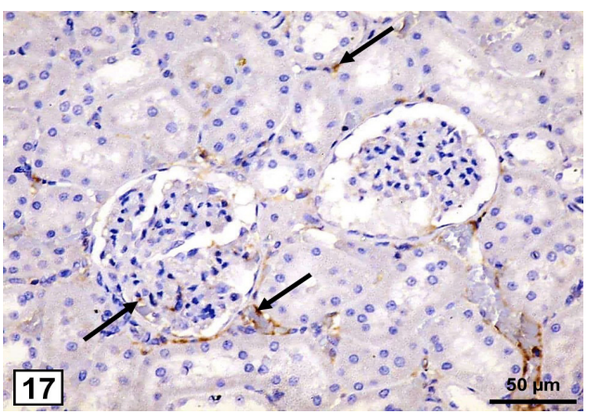
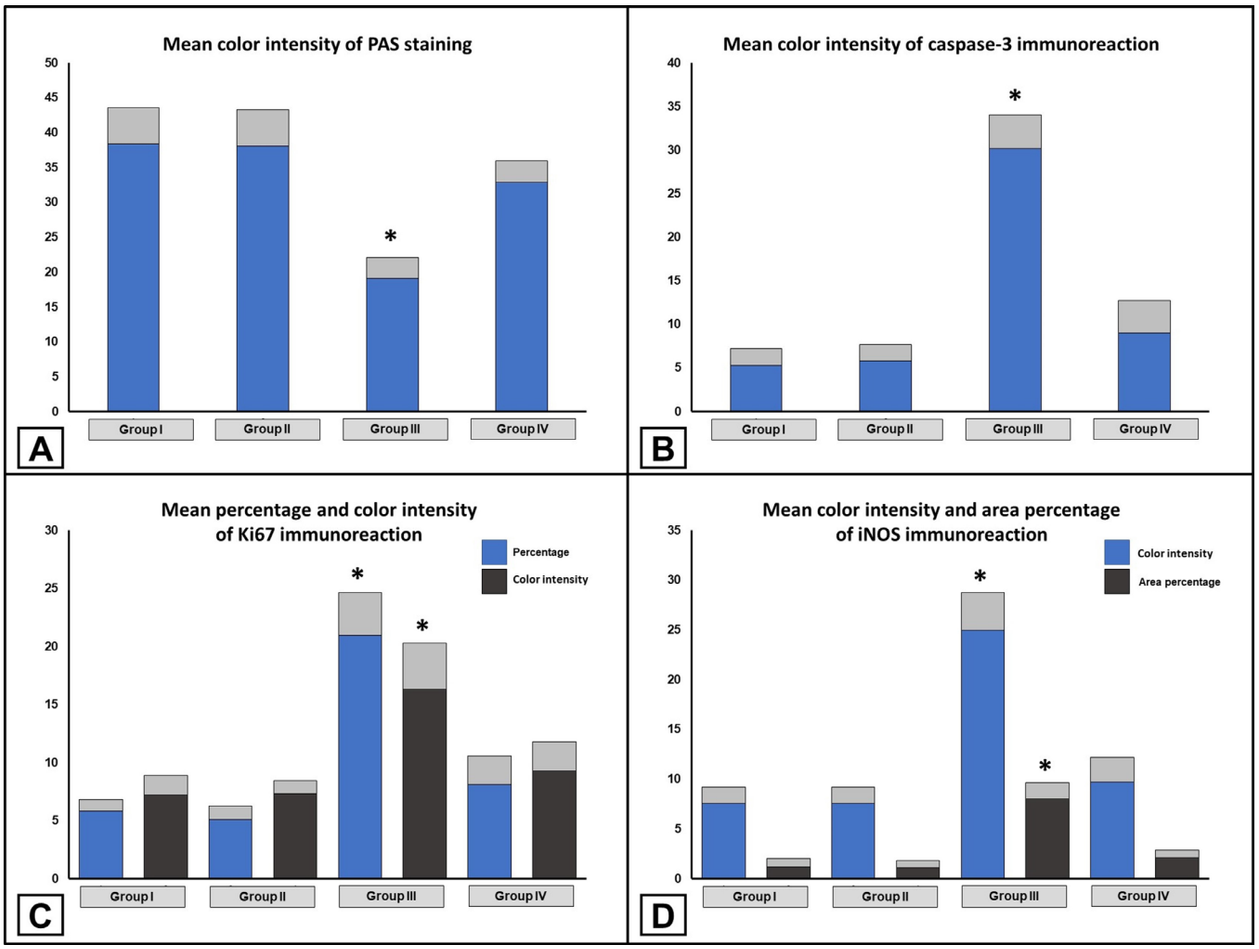


Fig.17: Group IV shows a moderate cytoplasmic iNOS immunoreaction in some glomerular (thin arrow) and interstitial cells (thick arrows). [iNOS x400, scale bar=50µm]

Table 1: Biochemical and morphometric analysis of the study groups

	Group I	Group II	Group III	Group IV
Mean tissue MDA level (nmol/g-tissue protein)	52.37±4.12	52.81±4.03	94.58±8.19^{a,b}	60.08±7.59^{c}
Mean color intensity of PAS histochemical staining	38.35±5.28	38.11±5.20	19.12±2.97^{a,b}	32.91±3.03^{c}
Mean color intensity of caspase-3 positive immunoreaction	5.29±1.93	5.82±1.84	30.17±3.88^{a,b}	9.05±3.72^{c}
Mean percentage of Ki67-positive cells	5.79±1.05	5.09±1.15	20.98±3.67^{a,b}	8.13±2.41^{c}
Mean color intensity of Ki67 positive cells	7.20±1.66	7.34±1.09	16.31±3.95^{a,b}	9.27±2.50^{c}
Mean color intensity of iNOS positive immunoreaction	7.58±1.59	7.54±1.66	24.94±3.81^{a,b}	9.73±2.43^{c}
Mean area percentage of iNOS positive immunoreaction	1.19±0.8	1.11±0.7	8.03±1.64^{a,b}	2.07±0.8^{c}

Significance is indicated by the superscript letters a,b,c against groups I, II and III respectively



Histogram 1: Morphometrical analysis of A] Mean color intensity of PAS histochemical staining, B] Mean color intensity of caspase-3 positive immunoreaction, C] Mean percentage and color intensity of Ki67-positive immunoreaction, and D] Mean color intensity and area percentage of iNOS positive immunoreaction. * denotes a significant difference versus control.

DISCUSSION

In this work, deferasirox-treated group recorded significant higher levels of the pro-oxidative marker, tissue MDA compared to control group. This finding was in consistence with a previous study[20]. They suggested that deferasirox induced oxidative stress with production of oxygen free radicals (ROS) which is a crucial factor in the occurrence of renal toxicity.

In the current study, the renal cortex, particularly the tubules, expressed numerous histological alterations with signs of inflammation in deferasirox-treated group. These findings were similarly reported in earlier studies[20,21]. The later related these damages to oxidative stress resulting after the use of deferasirox. Additionally, it was proved that deferasirox promotes an intense engorgement of mitochondria and suppresses the cellular ATP[3,22], which could limit transport processes and explains the proximal tubular dysfunction[23]. Moreover, as the intracellular iron proves indispensable for oxidative phosphorylation and production of ATP for the proper functioning of tubular cells[24], therefore, excessive rapid chelation of iron by deferasirox could explain the tubular damage[3,25]. Another proposed mechanism for proximal tubular injury induced by deferasirox is related to the increased excretion of urinary protein and glucose[21].

Caspase-3 is a main player in the intrinsic mitochondrial-mediated intrinsic apoptotic pathway. Caspase-3 is an effector caspase that causes alterations in the shape and biochemistry of apoptotic cells[13,24]. In the present work, the renal cortical histological changes were coupled with a significant upregulation in the mean color intensity of caspase-3 expression in deferasirox-treated group. Similarly, a previous study proved that deferasirox induced apoptosis in cultured tubular cells[21]. Another study reported that deferasirox directly insulted cultured proximal tubular cells and induced mitochondrial dysfunction and cellular death with blended characteristics of apoptosis and necrosis[21].

Meanwhile, in this study, the upregulation of caspase-3 activity was coupled with a significant rise in the expression of proliferating cells detected by Ki67 immunostaining in the renal tubules and corpuscles after

deferasirox administration. This finding could be attributed to the increase in ROS production by deferasirox leading to cellular proliferation as a cellular response to damaging organelles and DNA breaks[26], where it was reported that caspases activate a type of compensatory proliferation, known as apoptosis-induced proliferation[27].

Furthermore, in this study, a significant increase in iNOS expression was detected in deferasirox-treated group along with evident signs of inflammation. Similarly, Gómez-Guerrero *et al*.[28] reported the upregulation of iNOS renal expression in a rat model of immune glomerulonephritis associated with proliferation and inflammatory infiltration. They suggested a role for nitric oxide (NO) in the pathogenesis, yet the function of NO in the kidney is complicated. It can either reduce or aggravate renal injury, hanging on the equilibrium between its favorable hemodynamic role and its noxious effects[29], which explains the weak expression of iNOS in the renal cortical tubules in the deferasirox-treated group compared to the glomeruli of the same group. Moreover, an earlier study suggested that chronic suppression of iNOS could induce an increase in the renal cortical activity[30], thus could explain the increased rate of proliferation in the deferasirox-treated group.

Vitamin E is a strong antioxidant and anti-inflammatory agent with low cost and rare side-effects. It is considered as a promising therapy against acute kidney injury[10]. It was clinically reported that supplementing a high oral dose of vitamin E for 12 weeks amongst diabetic patients had beneficial effects on reducing oxidative stress and inflammation in the kidney[31].

In this work, the concomitant administration of vitamin E with deferasirox evidently restored the tissue MDA level, reduced the renal cortical histological changes, and improved the expression of the different immunohistochemical markers of apoptosis, proliferation, and inflammation. These results were similar to the work of other researchers[12,32]. Since vitamin E is a cell membrane hydrophobic compound[33], it is one of the main endogenous antioxidants that reduces lipid peroxidation and targets free radicals, thus protecting mitochondrial membranes from damage by ROS[33,34]. Scavenging oxygen radicals and preventing lipid peroxidation with vitamin E help to maintain renal glomerular structure and function[35].

Moreover, some authors reported the antiapoptotic protective role of vitamin E and its capability of downregulating caspase-3 immunoexpression with the subsequent restoration of the normal rate of cell proliferation and relieving of the inflammatory signs[36]. The role of vitamin E in maintaining cellular proliferation was previously suggested both in *vivo* and in *vitro*, where it was proposed to owe to the enhancement of the relevant gene expression[37,38].

Additionally, the role of vitamin E in suppressing tissue inflammation was suggested to be related to the depression of C-reactive protein and inhibiting the release of relevant proinflammatory cytokines and interleukin-6[39,40]. Furthermore, vitamin E induces its anti-inflammatory effect in various cell types through inhibiting the COX-2 and 5-LUX mediated eicosanoids and suppressing the NF-κB signaling pathways[41].

CONCLUSION

Deferasirox administration led to histological alterations in the renal cortical tubules through inducing oxidative stress, apoptosis, proliferation, and inflammation. Concomitant supplementation with vitamin E exerted an evident protective action against deferasirox harmful effects most probably through its antioxidative, antiapoptotic, and anti-inflammatory properties. It is recommended to proceed with more studies that include ultrastructural examination for more insight of the mechanism of the effect of deferasirox on the kidney in order to approach the best protective agent against its side effects.

CONFLICT OF INTERESTS

There are no conflicts of interest.

REFERENCES

1. Cunningham MJ. Update on Thalassemia: Clinical Care and Complications. Hematol Oncol Clin North Am 2010;24(1):215–27. https://doi.org/10.1016/j.hoc.2009.11.006.
2. Hershko C. Pathogenesis and management of iron toxicity in thalassemia. Ann N Y Acad Sci 2010;1202(1):1–9. https://doi.org/10.1111/j.1749-6632.2010.05544.x.
3. Scoglio M, Cappellini MD, D'Angelo E *et al*. Kidney Tubular Damage Secondary to Deferasirox: Systematic Literature Review. Child (Basel, Switzerland) 2021;8(12):1104. https://doi.org/10.3390/children8121104.
4. Bollig C, Schell LK, Rücker G *et al*. Deferasirox for managing iron overload in people with thalassaemia. Cochrane Database Syst Rev 2017;8(8):CD007476–CD007476. https://doi.org/10.1002/14651858.CD007476.pub3.
5. Badeli H, Baghersalimi A, Eslami S *et al*. Early Kidney Damage Markers after Deferasirox Treatment in Patients with Thalassemia Major: A Case-Control Study. Oxid Med Cell Longev 2019;2019:5461617. https://doi.org/10.1155/2019/5461617.
6. Viprakasit V, Ibrahim H, Ha S-Y *et al*. Clinical efficacy and safety evaluation of tailoring iron chelation practice in thalassaemia patients from Asia-Pacific: a subanalysis of the EPIC study of deferasirox. Int J Hematol 2011;93(3):319–28. https://doi.org/10.1007/s12185-011-0789-8.
7. Yui JC, Geara A, Sayani F. Deferasirox-associated Fanconi syndrome in adult patients with transfusional iron overload. Vox Sang 2021;116(7):793–7. https://doi.org/10.1111/vox.13064.

8. Aggarwal BB, Sundaram C, Prasad S, Kannappan R. Tocotrienols, the vitamin E of the 21st century: its potential against cancer and other chronic diseases. Biochem Pharmacol 2010;80(11):1613–31. https://doi.org/10.1016/j.bcp.2010.07.043.
9. Galli F, Bonomini M, Bartolini D *et al*. Vitamin E (Alpha-Tocopherol) Metabolism and Nutrition in Chronic Kidney Disease. Antioxidants 2022;11(5):989. https://doi.org/10.3390/antiox11050989.
10. Liu P, Feng Y, Wang Y, Zhou Y, Zhao L. Protective effect of vitamin E against acute kidney injury. Biomed Mater Eng 2015;26(s1):S2133–44. https://doi.org/10.3233/bme-151519.
11. Rezaei Y, Khademvatani K, Rahimi B, Khoshfetrat M, Arjmand N, Seyyed-Mohammadzad M-H. Short-Term High-Dose Vitamin E to Prevent Contrast Medium-Induced Acute Kidney Injury in Patients With Chronic Kidney Disease Undergoing Elective Coronary Angiography: A Randomized Placebo-Controlled Trial. J Am Heart Assoc 2016;5(3):e002919–e002919. https://doi.org/10.1161/JAHA.115.002919.
12. Zhao Y, Zhang W, Jia Q *et al*. High Dose Vitamin E Attenuates Diabetic Nephropathy via Alleviation of Autophagic Stress. Front Physiol 2019;9:1939. https://doi.org/10.3389/fphys.2018.01939.
13. Fang J, Xie S, Chen Z *et al*. Protective Effect of Vitamin E on Cadmium-Induced Renal Oxidative Damage and Apoptosis in Rats. Biol Trace Elem Res 2021;199(12):4675–87. https://doi.org/10.1007/s12011-021-02606-4.
14. Anderson CP, Shen M, Eisenstein RS, Leibold EA. Mammalian iron metabolism and its control by iron regulatory proteins. Biochim Biophys Acta 2012;1823(9):1468–83. https://doi.org/10.1016/j.bbamcr.2012.05.010.
15. Gaertner DJ, Hallman TM, Hankenson FC, Batchelder MA. Anesthesia and analgesia for laboratory rodents. In: Fish, R.E., Danneman, P.J., Brown, M., Karas AZ, ed. Anesth. Analg. Lab. Anim. 2nd ed.Elsevier Academic Press, London (UK); 2008;239–97. https://doi.org/10.1016/b978-012373898-1.50014-0.
16. Kurokawa T, Itagaki S, Yamaji T *et al*. Antioxidant Activity of a Novel Extract from Bamboo Grass (AHSS) against Ischemia-Reperfusion Injury in Rat Small Intestine. Biol Pharm Bull 2006;29(11):2301–3. https://doi.org/10.1248/bpb.29.2301.
17. Gamble M. The Hematoxylins and Eosin. In: Bancroft, JD and Gamble M, ed. Theory Pract. Histol. Tech. 6th ed.Philadelphia: Churchill Livingstone Elsevier; 2008;121–34. https://doi.org/10.1016/b978-0-443-10279-0.50016-6.
18. Myers R, Fredenburgh J, Grizzle W. Carbohydrates. In: Bancroft J and GM, ed. Theory Pract. Histol. Tech. 6th editioChurchill Livingstone Elsevier; 2008;161–86.
19. Buchwalow IB, Böcker W. Working with Antibodies. Immunohistochem. Basics Methods. Springer Berlin Heidelberg; 2010;31–9. https://doi.org/10.1007/978-3-642-04609-4_4.
20. Fereydouni T, Hajihashemi S, Yousefichaijan P, Rahbari A. Protective Effects of Vitamin C Concomitant Treatment on Deferasirox-induced Renal Toxicity in Rats. J Arak Univ Med Sci 2021;23(6):926–43. https://doi.org/10.32598/jams.23.6.62.7.
21. Sánchez-González PD, López-Hernandez FJ, Morales AI, Macías-Nuñez JF, López-Novoa JM. Effects of deferasirox on renal function and renal epithelial cell death. Toxicol Lett 2011;203(2):154–61. https://doi.org/10.1016/j.toxlet.2011.03.018.
22. Gottwald EM, Schuh CD, Drücker P *et al*. The iron chelator Deferasirox causes severe mitochondrial swelling without depolarization due to a specific effect on inner membrane permeability. Sci Rep 2020;10(1):1577. https://doi.org/10.1038/s41598-020-58386-9.
23. Paul BT, Manz DH, Torti FM, Torti S V. Mitochondria and Iron: current questions. Expert Rev Hematol 2017;10(1):65–79. https://doi.org/10.1080/17474086.2016.1268047.
24. Martin-Sanchez D, Gallegos-Villalobos A, Fontecha-Barriuso M *et al*. Deferasirox-induced iron depletion promotes BclxL downregulation and death of proximal tubular cells. Sci Rep 2017;7:41510. https://doi.org/10.1038/srep41510.
25. Kattamis A. Renal function abnormalities and deferasirox. Lancet Child & Adolesc Heal 2019;3(1):2–3. https://doi.org/10.1016/s2352-4642(18)30350-x.
26. Yumuşak N, Koca G, Akbulut A, Atilgan Hİ, Korkmaz M. Proliferative and apoptotic evaluations of renal preventive effects of coenzyme Q10 in radioiodine-131 induced renal damage. Ankara Üniversitesi Vet Fakültesi Derg 2021. https://doi.org/10.33988/auvfd.871118.
27. Fogarty CE, Bergmann A. Killers creating new life: caspases drive apoptosis-induced proliferation in tissue repair and disease. Cell Death Differ 2017;24(8):1390–400. https://doi.org/10.1038/cdd.2017.47.
28. Gómez-Guerrero C, López-Franco O, Suzuki Y *et al*. Nitric oxide production in renal cells by immune complexes: Role of kinases and nuclear factor-κB. Kidney Int 2002;62(6):2022–34. https://doi.org/10.1046/j.1523-1755.2002.00653.x.
29. Yoon G, Oh CS, Kim H-S. Hypergravity upregulates renal inducible nitric oxide synthase expression and nitric oxide production. Oncotarget 2016;7(21):30147–54. https://doi.org/10.18632/oncotarget.9253.

30. Martin B, Caron N, Jadot I *et al*. Evaluation of inducible nitric oxide synthase inhibition on kidney function and structure in high-fat diet-induced kidney disease. Exp Physiol 2018;103(1):125–40. https://doi.org/10.1113/EP086594.

31. Khatami PG, Soleimani A, Sharifi N, Aghadavod E, Asemi Z. The effects of high-dose vitamin E supplementation on biomarkers of kidney injury, inflammation, and oxidative stress in patients with diabetic nephropathy: A randomized, double-blind, placebo-controlled trial. J Clin Lipidol 2016;10(4):922–9. https://doi.org/10.1016/j.jacl.2016.02.021.

32. Eid RA, Zaki MSA, Alghamd MA *et al*. Ameliorative Effect of Vitamin E on Biochemical and Ultrastructural Changes in Artemether-induced Renal Toxicity in Rats. Int J Morphol 2020;38(2):461–71. https://doi.org/10.4067/s0717-95022020000200461.

33. Ostróżka-Cieślik A. The Effect of Antioxidant Added to Preservation Solution on the Protection of Kidneys before Transplantation. Int J Mol Sci 2022;23(6):3141. https://doi.org/10.3390/ijms23063141.

34. Ghlissi Z, Hakim A, Mnif H *et al*. Combined use of Vitamins E and C improve nephrotoxicity induced by colistin in rats. Saudi J Kidney Dis Transplant 2018;29(3):545. https://doi.org/10.4103/1319-2442.235168.

35. Attia DM, Ni ZN, Boer P *et al*. Proteinuria is preceded by decreased nitric oxide synthesis and prevented by a NO donor in cholesterol-fed rats. Kidney Int 2002;61(5):1776–87. https://doi.org/10.1046/j.1523-1755.2002.00313.x.

36. Shoukry HS, Hassanien RT, Rasheed RA, Kamel MM, Ibrahim ER, Ibrahim HS. Vitamin E Improves Doxorubicin Induced Nephrotoxicity; Possible Underlying Mechanisms. Med J Cairo Univ 2018;86(March):651–7. https://doi.org/10.21608/mjcu.2018.55380.

37. Rahimi Anbarkeh F, Nikravesh MR, Jalali M, Sadeghnia HR, Sargazi Z. The Effect of Diazinon on Cell Proliferation and Apoptosis in Testicular Tissue of Rats and The Protective Effect of Vitamin E. Int J Fertil Steril 2019;13(2):154–60. https://doi.org/10.22074/ijfs.2019.5612.

38. Ki Hoon Ahn, Hwa Kyung Jung, So Eun Jung, Kyong Wook Yi HTP, Jung Ho Shin, Young Tae Kim, Jun Young Hur, Sun Haeng Kim TK. Microarray Analysis of Gene Expression During Differentiation of Human Mesenchymal Stem Cells Treated with Vitamin E in *vitro* into Osteoblasts. Korean J Bone Metab 2011;18(1):23–32.

39. Nazrun AS, Norazlina M, Norliza M, Nirwana SI. The anti-inflammatory role of vitamin e in prevention of osteoporosis. Adv Pharmacol Sci 2012;2012:142702. https://doi.org/10.1155/2012/142702.

40. Asbaghi O, Sadeghian M, Nazarian B *et al*. The effect of vitamin E supplementation on selected inflammatory biomarkers in adults: a systematic review and meta-analysis of randomized clinical trials. Sci Rep 2020;10(1):17234. https://doi.org/10.1038/s41598-020-73741-6.

41. Jiang Q. Natural forms of vitamin E: metabolism, antioxidant, and anti-inflammatory activities and their role in disease prevention and therapy. Free Radic Biol Med 2014;72:76–90. https://doi.org/10.1016/j.freeradbiomed.2014.03.035.

الملخص العربى

الدور الوقائي المحتمل لفيتامين (هـ) ضد الإصابة التي يسببها عقار ديفيراسيروكس لأنابيب القشرة الكلوية في ذكور الجرذان البيضاء البالغة: دراسة نسيجية وهستوكيميائية مناعية

شيماء محمود عبد الحليم بدر و هبة السيد محمد شرف الدين و مروة عوض عبد الحميد إبراهيم

قسم الهستولوجيا و بيولوجيا الخلية ـ كلية الطب ـ جامعة طنطا

مقدمة: ديفيراسيروكس ، باعتباره عقار يستخدم لاستخلاب الحديد و يعطى عن طريق الفم، يعتبر حاليًا خط العلاج الأول للحمل الزائد للحديد الناجم عن عمليات نقل الدم المتكررة نظرًا لسهولة استخدامه وفعاليته وتوافره البيولوجي العالي، ومع ذلك فقد تم تسجيل حدوث إصابة الكلى الحادة والخلل الأنبوبي مع استخدامه. فيتامين (هـ) هو أحد مضادات الأكسدة القوية وعامل مضاد للالتهابات.

الهدف من العمل: دراسة تأثير عقار ديفيراسيروكس على حالة التأكسد و التركيب النسيجي ،و موت الخلايا المبرمج و تكاثر الخلايا والالتهاب لأنابيب القشرة الكلوية ودراسة الدورالوقائي المحتمل لفيتامين (هـ) ضد هذا التأثير في ذكور الجرذان البيضاء البالغة.

مواد و طرق البحث: تم تقسيم أربعة وعشرين من ذكور الجرذان البيضاء إلى أربع مجموعات متساوية. المجموعة الضابطة, المجموعة المعالجة بفيتامين (هـ) ، المجموعة المعالجة بديفيرازيروكس (١٠٠ مجم / كجم / يوم من ديفيراسيروكس لمدة ٤ أسابيع) ، المجموعة المعالجة بفيتامين (هـ) مع ديفيرازيروكس (تتناول فيتامين (هـ) وديفيرازيروكس بشكل متزامن). ثم تمت معالجة عينات الكلى للدراسات الكيميائية الحيوية والنسيجية والهستوكيميائية المناعية المختلفة.

النتائج: كشفت المجموعة المعالجة بـديفيراسيروكس عن فقدان البنية النسيجية الطبيعية للقشرة الكلوية مع العديد من التغيرات النووية والسيتوبلازمية للأنابيب مع وجود علامات الالتهاب النسيجي. ارتبط ارتفاع ذو دلالة احصائية في مستوى المالونالدهيد النسيجي بارتفاع ذو دلالة احصائية في التعبير الهستوكيميائي المناعي لكل من caspase-٣ و Ki٦٧ و iNOS ، بينما ظهر انخفاض في التعبير الهستوكيميائي لصبغة. PAS أوضحت نتائج المجموعة المعالجة بفيتامين (هـ) وديفيرازيروكس بشكل متزامن نسيجًا طبيعيًا ظاهريًا للقشرة الكلوية مع اختلاف غير ذي دلالة احصائية في جميع المعلمات المدروسة مقارنةً بالمجموعة الضابطة.

الاستنتاج: أدى العلاج بعقار ديفيراسيروكس إلى تغيرات نسيجية في أنابيب القشرة الكلوية من خلال إحداث الإجهاد التأكسدي ،و موت الخلايا المبرمج و تكاثر الخلايا والالتهاب.و أظهر فيتامين (هـ) كمكمل مصاحب تأثيرًا وقائيًا ضد آثار ديفيراسيروكس الضارة على الأرجح من خلال خصائصه المضادة للأكسدة ومضادات موت الخلايا المبرمج والمضادة للالتهابات.