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Effect of Tenofovir Administration and Withdrawal on the Kidney Tissues of Adult Male Albino Rats and the Protective Role of Vitamin E

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Abstract

Tenofovir disoproxil fumarate is one of antiretroviral drugs used to treat chronic hepatitis B and HIV. In recent studies many Tenofovir problems were recorded; acute kidney injury (AKI), chronic kidney diseases (CKD) and Fanconi syndrome. Vitamin E is a powerful antioxidant that reduce free radical damage. Vitamin E supplementation can protect kidney tissues and enhances their regeneration if it is added to Tenofovir treatment.

The aim of the present study is to describe the changes which occur in the kidney tissues of the adult male albino rats after Tenofovir administration and determine whether these changes are reversible or not after drug withdrawal, Also, whether vitamin E has a protective role or not.

A total number of 40 adult male albino rats weighting 200-250 grams were used. Rats were divided into four groups, 10 rats in each group. The first group I (control group), The second group II which was treated with Tenofovir 10 mg/kg/day for two months, the third group III which was treated with Tenofovir10 mg/kg/day for two months, vitamin E also was given 100mg/kg/day for two months. The fourth group IV which was treated with Tenofovir 10 mg/kg/day for two months then withdrawal of Tenofovir for one month.

There was marked degeneration of renal glomeruli and tubules in group II which was highly statistically significant than control group (P< 0.001), while in group III there was marked improvement of renal glomeruli and tubules which was highly statistically significant than group II (P<0.001). while in group IV these changes were moderately significant when compared with group II (P<0.01). Therefore, Vitamin E has a protective role if it is added to Tenofovir treatment, also vitamin E enhances regeneration of renal tissues after Tenofovir withdrawal.

Keywords: Tenofovir, Kidney, Fanconi syndrome, Vitamin E.

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Introduction

Kidneys are highly vascularized tubular glands formed from numerous, closely packed uriniferous tubules (Bacha and Bacha, 2012). Kidneys play several critical roles in maintaining fluid and regulating electrolytes balance. They produce and activate hormones that are involved in calcium metabolism, erythrogenesis and regulation of blood pressure (Findlay and Isles, 2015). Tenofovir disoproxil fumarate (TDF) is one of the antiretroviral drugs. It has been widely used for long-term treatment of HIV and chronic hepatitis B (CHB) infections in adult patient (pham and Gallant, 2006). Tenofovir disoproxil fumarate (TDF) is an orally bioavailable pro-drug of Tenofovir (TFR), an acyclic nucleotide analogue reverse- transcriptase inhibitor (NRTI) (Buti et al., 2012). Tenofovir prevents HIV reverse transcriptase through competition with natural substrate deoxyadenosine 5'-triphosphate after incorporation into DNA. (Lyseng - Williamson et al., 2005). Long term use of Tenofovir is associated with nephrotoxicity and bone loss. Presentation of nephrotoxicity can appear as Fanconi syndrome, acute kidney injury, or decline of glomerular filtration rate (GFR) (Ustianowski et al., 2015). The exact mechanism of TDF -induced nephrotoxicity is not clear but is thought to be related to the accumulation of TDF in the proximal tubules. Studies have shown that Tenofovir mainly taken up from the blood into proximal tubules cells by human organic anion transporter 1(HOAT1) at the basolateral membrane (Cihlar et al., 2001). Vitamin E is a group of fat-soluble compounds with antioxidant activities, Serum concentrations of vitamin E (alpha-tocopherol) depend on the liver which takes up the nutrient after the various forms are absorbed from the small intestine. The liver re-secretes only alpha-tocopherol via the hepatic alpha-tocopherol transfer protein (Traber, 2006).

Vitamin E is a lipid soluble vitamin and therefore over 90% of total body vitamin E is found in the adipose tissue (Meydani et al., 1996). Vitamin E prevents reactive oxygen species which spread through biological membranes or fat when lipid content is oxidized by reacting with lipid radicals to give more stable products (Choe et al. 2009). Oxidative stress induced by the production of reactive oxygen species (ROS) during activation of polymorphonuclear leukocytes and macrophages. This is commonly occurred in HIV patients and can lead to more replication of the virus (Das et al., 1990). Supplementation of Vitamin E decreased oxidative stress and altered kidney function markers if it is added to Tenofovir treatment (Oluwatosin et al. 2012). The present study is designed to detect the effects of Tenofovir administration and withdrawal on the kidney tissues of Adult Wistar albino rats and to detect whether vitamin E has a protective role or not.

Materials and methods

Tenofovir was purchased from Seif Co. for chemicals and pharmaceuticals, Cairo, Egypt. Vitamin E was obtained from Pharco Company for chemicals, Cairo, Egypt.

Animals and experimental design

Forty adult male Wistar albino rats (weighing about 200-250 g and aged from 3-4 months) were obtained from the animal house, Faculty of Medicine, Assuit University and housed in a special wire cages and kept under complete isolated conditions in the animal facilities of Faculty of Medicine, Sohag University for two weeks prior to experiment for adaptation. Animals were kept on a 12-hour light, 12-hour dark cycle with ambient temperature of 22-24 °C and were provided a commercial pelleted feed and water ad libitum. Experimental design and all animal handling procedures were approved by the Research Ethical Committee of the Faculty of Veterinary Medicine, South Valley University, Qena, Egypt (the approval no.

20147). All precautions were taken into consideration to avoid animal suffering.

Experimental design

After acclimation, forty rats were randomly assigned to four different isolated groups (Ten animals each); group I was administered normal saline and act as a control group. Group II was received Tenofovir at a dose of 10 mg/ kg/day for two months by stomach gavage. Group III was received Tenofovir at a dose of 10 mg/kg/day and vitamin E also was given 100mg/kg/day for two months. Group IV was received Tenofovir10 mg/kg/day for two months then withdrawal of Tenofovir for one month.

Methods

Renal tissue sampling: Twenty-four hours after the last treatment, rats were sacrificed by decapitation and kidneys were rapidly excised from each animal, divided equally and washed with 0.9% NaCl solution and distilled water, then blotted using filter paper. One portion was fixed in 10% neutral buffered formalin for histopathological examination and the other part was kept freeze under - 20 ℃ for peroxidation assays. The sampling process was performed after two months except control group (group I) where all rats were sacrificed at day 10th. Renal samples were washed with a phosphate buffered saline (PBS) solution, pH 7.4 including 0.16 mg /ml heparin to get rid of red blood cells and clots preceding its dissection into specimen.

Electron microscopic examination technique: Using a sharp knife, each kidney was cut in a sagittal plane into two halves and small cubes were taken and kept in 5 % glutaraldehyde in sodium cacodylate buffer at 4℃ for 24-48 hours. Samples were washed in sodium cacodylates buffer at pH 1.5 for three changes 20 minutes each Samples were post fixed in 1% osmium tetroxide for hours. Samples were washed in four changes of sodium cacodylates buffer, 20 minutes each. Samples were dehydrated in ascending grades of alcohol as follows 30% for 30 minutes, 50% for 30

minutes, 70% over night in a refrigerator 90% for 30-60 minutes and then in 100% twice 30 minutes each. Samples then were put in the following solutions: Propylene oxide for 30-60 minutes, propylene oxide placed in Epons-Araldite formulation (1:1) over night, pure Epon- Araldite formulations for 30-60 minutes. The tissues were embedded in a pure Epon-Araldite mixture for 24 hours (Ayache et al., 2010). Tissues blocks were polymerized in an oven at 35℃ for 24 hours, at 45℃ for 24 hours and at 60℃ for 24 hours. Two types of section cutting were done:

a) Semi thin sectioning at 1umSpecimens were trimmed with a razor blade and stained with 2 % aqueous toluidine blue then dried by absolute ethanol on a hot plate at 40℃.

b) Ultrathin sectioning at 50 nm were recommended on cooper grids examined by a transmission Jeol=JEM=100CXII electron microscope and photographed at the electron microscope unit of Assiut university.

Alkaline phosphatase reaction by Immuno-histochemical technique

Paraffin sections were cut into 5 µm thick slices. After paraffin was removed using xylene, the sections were incubated in 0.3% hydrogen peroxide in absolute methanol for 30 min at room temperature in order to inactivate endogenous peroxidase. The sections were then washed with 0.01M phosphate buffer, pH 7.2 containing 0.85% sodium chloride (PBS) and 10% normal horse serum was placed on the sections for 20 min at room temperature. After washing with PBS, anti-rat kidney ALP rabbit diluted 50-fold with PBS was applied to the sections and left for 30 min at room temperature (Chida, 1993). Thereafter, the sections were washed with PBS and incubated with an avidin-biotin-peroxidase complex (ABC) kit (Vectastain ABS kit; Vector Laboratories, Burlingame,CA).

Morphometric &Statistic analysis

Estimation of the diameters of the glomeruli, proximal and distal convoluted tubules (maximum diameter) in the control and treated groups (at magnification of 400). The previous parameters were measured using Digimizer Image Analysis Program (MedCalc Software Co). Statistical analysis: Variables were represented by mean ± SD (Mean ± standard deviation of the mean).

Results

Table 1: Effect of Tenofovir (10 ml/kg), vitamin E (100 mg/kg), and their combination (Tenofovir + vitamin E) and withdrawal on glomerular, proximal and distal tubular diameter after two months of experiment were estimated from the paraffin sections analysis system (Leica Q500 MC). (Mean ± SE) (N =10)

|	**Group I**	**Group II**	**Group III**	**Group IV**
Glomeruli	102.74 ±7.9	79.57±8.8***	100.68±2.8***	88.48±5.5**
PCT	44.51±1.8	29.75±5.12***	41.14±2.44***	33.94±2.5**
DCT	33.33±3.12	25.68±2.11***	32.37±2.05***	29.44±1.01**

Alkaline phosphatase reaction

In control group positive reaction in the form of dark brown granules in the apical surface of proximal convoluted tubules (Fig. 3 a). In group II: negative Alkaline phosphatase reaction (Fig. 3 b). In group III: positive Alkaline phosphatase reaction (Fig.3 c). In group IV: Minimal positive Alkaline phosphatase reaction was observed in the form of minimal dark brown granules (Fig. 3 d).

Morphometric results: Mean glomerular diameter in control group was 102.74 ±7.9 while it was 79.57± 8.8 in group (II) which was marked decreased in comparison with control group while in group (III) it was 100.68± 2.8 which was nearby the diameter in control group. Glomerular diameter in group (IV) was 88.48±5.5.

Mean PCT diameter in control group was 44.51±1.8 while it was 29.75±5.12 in group (II) which was marked decreased in comparison with control group while in group (III) it was 41.14±2.44 which was nearby the diameter in control group. PCT diameter in group (IV) was 33.94±2.5.

Mean DCT diameter in control group was 33.33±3.12 while it was 25.68±2.11 in group (II) which was marked decreased in comparison with control group while in group (III) it was 32.37±2.05 which was nearby the diameter in control group, diameter in group (IV) was 29.44±1.01.
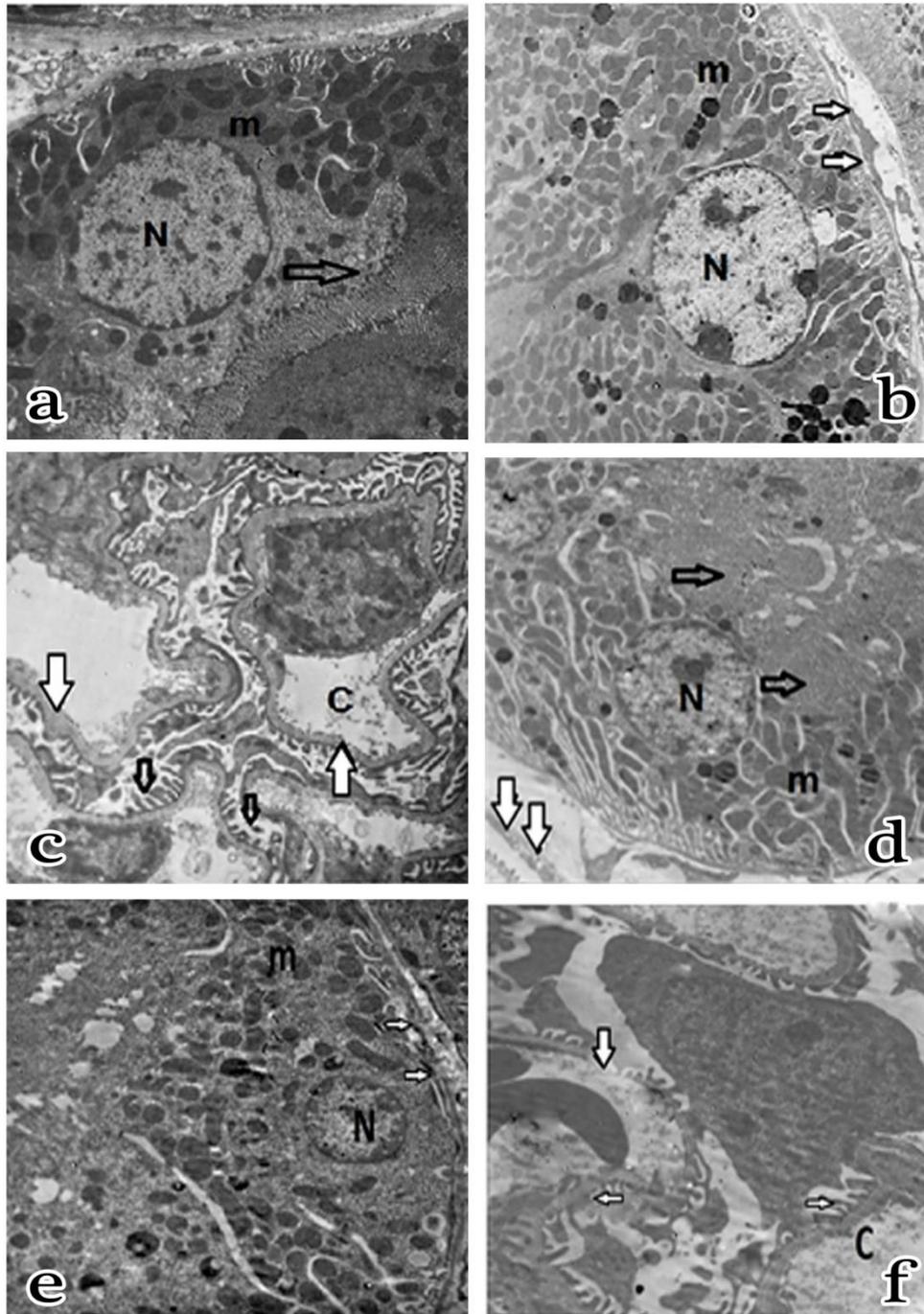
Fig. (1): Electro-photomicrographs of kidney sections from control and experimental group, represented, (Figs. a-c) normal control kidney sections in the proximal convoluted tubule from group I (Fig. a) showing normal rounded nucleus (N) and multiple elongated basally arranged mitochondria (m), regular apical microvilli (arrow). Distal convoluted tubule (fig. b) showing normal rounded nucleus (N) and multiple elongated basally arranged mitochondria (m) and regular basement membrane (Thick arrow), glomerular capillaries (Fig. c) showing glomerular capillary (C) The capillary endothelium rest on basal lamina (arrow head). The podocytes have multiple pedicles (arrows). group II (Fig. d, e, f). proximal convoluted tubule (Fig. d) showing shrunken nucleus (N), swollen mitochondria(m) with distortion of brush border(arrows), Distal convoluted tubule (Fig. e) showing shrunken nucleus (N), swollen mitochondria(m) with thickening of the basement membrane (Thick arrow). glomerular capillaries (Fig. f) showing irregular basal lamina in some areas (Thick arrow) and distorted pedicles (arrow). X5810
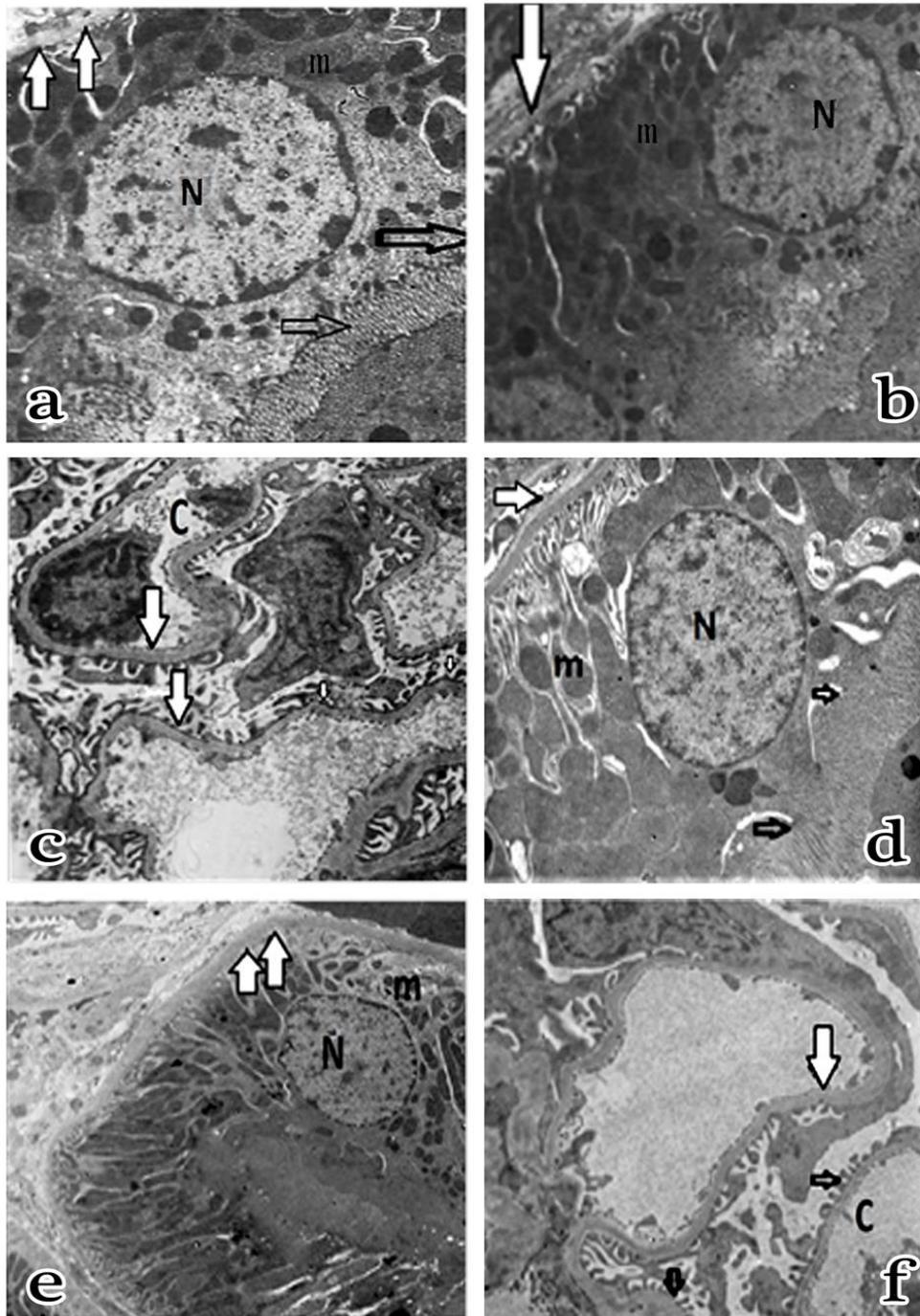
Fig. (2): Electro-photomicrograph of kidney sections from experimental groups, represented, (Figs. a-c) represented group III. proximal convoluted tubule from group III (Fig. a) showing normal rounded nucleus (N) and multiple elongated basally arranged mitochondria (m), regular apical microvilli (arrow). Distal convoluted tubule (fig. b) showing normal rounded nucleus (N) and multiple elongated basally arranged mitochondria (m) and regular basement membrane (Thick arrow), glomerular capillaries (Fig. c) showing glomerular capillary (C) The capillary endothelium rest on basal lamina (arrow head). The podocytes have multiple pedicles (arrows). group IV (Figs. d, e, f). proximal convoluted tubule (Fig. d) showing shrunken nucleus (N), swollen mitochondria(m) with distortion of brush border(arrows), Distal convoluted tubule (Fig. e) showing shrunken nucleus (N), swollen mitochondria(m) with thickening of the basement membrane (Thick arrow). glomerular capillaries (Fig. f) showing irregular basal lamina in some areas (Thick arrow) and distorted pedicles (arrow) X5810
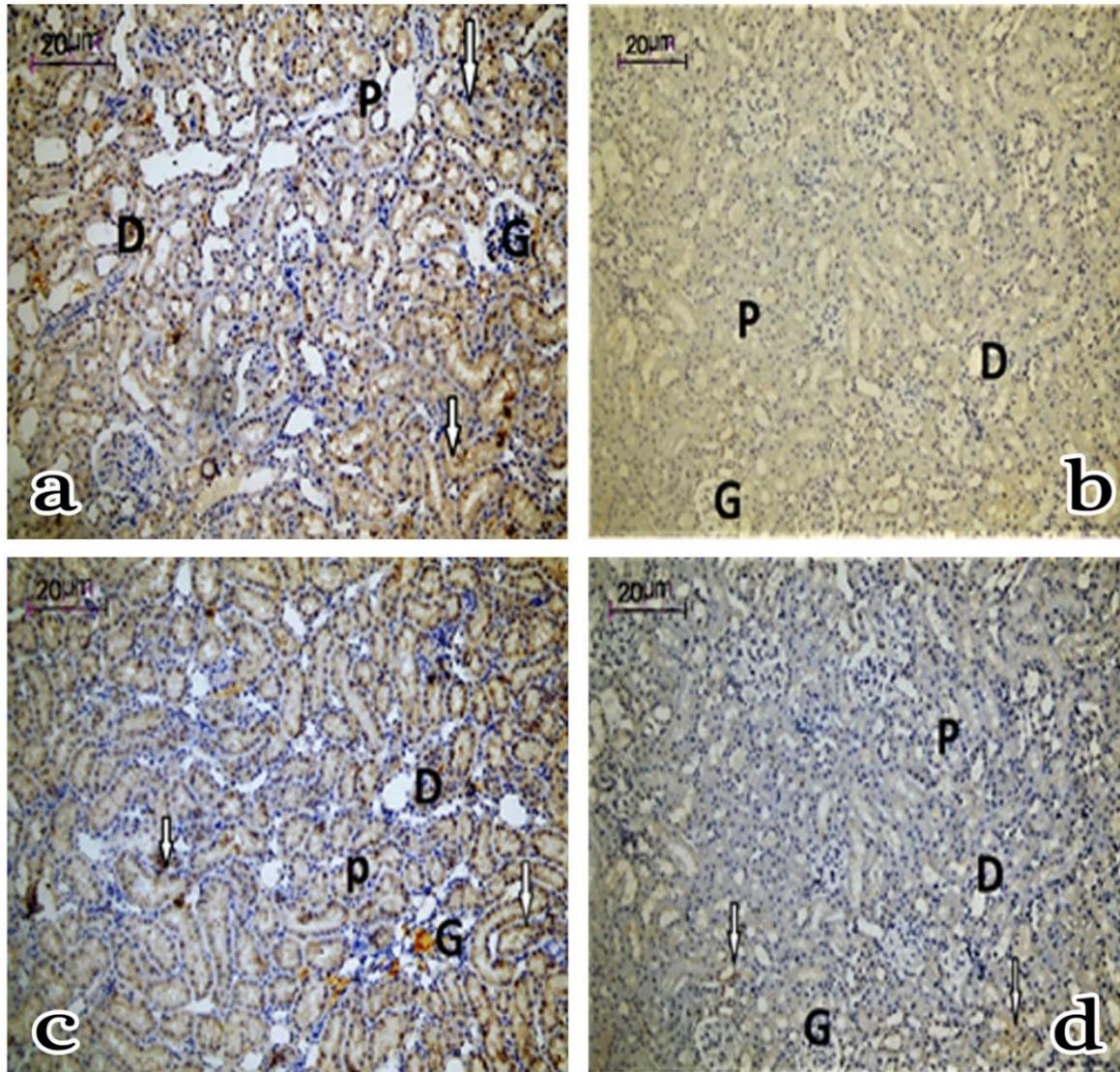
Fig. (3): Photomicrograph of kidney sections from control and experimental groups, represented Alkaline phosphatase reaction (Figs. a-d): normal control kidney sections (Fig. a) showing positive reaction in the form of dark brown granules in the apical part of proximal convoluted tubules (arrows). Group II (Fig. b) showing negative alkaline phosphatase reaction. Group III (Fig. c) showing positive reaction (arrows). Group IV (Fig. d) showing minimal positive reaction (arrows). Bars = 20 µm in all parts.

Discussion

In the present work electron microscopic examination showing: In group II there was shrunken nucleus, mitochondria were swollen and irregular in shape. There was distortion of brush border, thickening of basement membrane as compared with the control group. Renal glomeruli showed thickening in the basal lamina with distorted pedicles. These results coincide with James et al. (2009) who reported that mitochondrial ultra-structural changes in the form of increased mitochondrial

number, irregular shape were associated with the administration of TDF.

Alkaline phosphatase reaction was negative especially in the apical part of the proximal convoluted tubules when compared with control group. These results coincide with Jung et al. (2018) who reported several cases of renal tubular dysfunction and Fanconi syndrome in TDF-treated patients which lead to excessive urinary excretion of solutes handled by the proximal tubule such as phosphate, glucose, and bicarbonate.

As a result, hypophosphatemia was a possible complication in TDF treated patients. Pandit et al. (2012) explained that these changes were due to oxidative stress which caused loss of membrane fluidity, changes in membrane potential and an increase in membrane permeability. Another possible mechanism could be due direct kidney damage induced by TDF leading to dysfunction and disturbance in the biosynthesis evaluated biochemical parameters which lead to the elevation of serum concentrations of specific biochemical parameters; ALT, AST, ALP because of their release or secretions from the damaged tissues (Kuester et al., 2002).

In group III there was regular nucleus with regular nuclear membrane, Mitochondria were elongated basally located, PCTs showed normal brush border, glomerular capillaries showed regular basal lamina with regular multiple pedicles. These results coincide with Simona et al. (2015) who reported that vitamin E supplementation may be an effective accessory therapy to regulate superoxide generation in human mitochondrial ROS, monocytes and neutrophil, this protective effect on oxidative stress attenuates the onset and development of several chronic/degenerative diseases including CKD, Alkaline phosphatase reaction was positive when compared with group (II). These results were confirmed by Mine et al. (2009) reported that vitamin E supplementation increases alkaline phosphatase activity in the kidney.

In group IV there was some irregularity of nuclear membrane, swollen mitochondria with some distortion of brush border of PCTs, thickening of basement membrane at some areas, Glomerular capillaries showed thickening of basal lamina at some areas with pedicles distortion but less than group (II). These results were supported by Abhasnee et al. (2017) who detected improvement of urinary biomarkers after Tenofovir withdrawal which may represent an early sign of proximal tubular dysfunction recovery, Alkaline phosphatase reaction showed minimal positive reaction when compared with group (I) and (III).

In our study, morphometric results coincide with Post (2010) who reported that Tenofovir treated group had smaller renal corpuscle and glomerular diameter compared with control group. Also (Nel et al., 2017) explained that renal corpuscle diameter and renal space after TDF administration had smaller diameter compared with the control group suggesting that TDF decreased glomerular filtration rate possibly due to its effect on tubular function.

Conclusion

Tenofovir should be used with caution in patients with high risks of renal dysfunction and even a mild degree of renal dysfunction.

Acknowledgments

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