Original Article

Nephroprotective Effects Of Sildenafil Against Nephrotoxicity In Animal Model

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Abstract

Background: The nephroprotective drugs are having essential roles in reducing or preventing the possibility of toxicity with wide range of drugs used for severe infections or cancers and still now the researchers investigate many drugs with different mechanisms and examine their possibility to combined with nephrotoxic drugs in different ways in order to reduce their toxicity or prolong the limited duration of treatment. In same way, the present study designed to find the potential nephroprotective activity of sildenafil in reversing or reducing the nephropathies induced by gentamycin.

Methods: The experiment was designed to include thirty-two rats and these were divided into four groups. The first (control) group received isotonic saline (2mL/kg/day i.p.). Second (positive control) group were received only sildenafil (5 mg/kg/day orally p.o).Third (induction) group received gentamycin (100 mg/kg/day i.p.). Fourth (treatment) group received sildenafil (5mg/kg/day p.o.) followed by gentamycin (100 mg/kg/day i.p.). The course of treatment continued to ten consecutive days . All animal were anesthsized at day $11^{th.}$ and blood collected by special tubes by cardiac puncture in order to be used for investigating of serum creatinine, urea, total protein , albumin and kidney injury molecules-1(KIM-1). Also, the kidneys were harvested and kept in formalaldehyde 10% for evaluation of histopathological changes.

Results: The gentamycin (induction)group showed all features of nephrotoxicity by producing a significant elevation of serum creatinine, urea , and KIM-1levels, with a significant reduction in serum total protein and albumin levels. Furthermore, the histopathological changes proved the nephrotoxicity in compared to control group. While sildenafil (treatment) group displayed the ameliorating effect on serum biomarkers and histopathological changes in comparison with both control (non-significant changes) and induction group (significant changes) at level of significant 0.05%.

Conclusion; This study ended with conclusion, which can be summarized as potential nephroprotective of sildenafil in selected dose against gentamycin nephrotoxic dose in rat model.

keywords: Nephrotoxicity; Gentamycin; Sildenafil ,Rat.

Introduction

There are many limitations of the doses or periods of certain drugs belong to the specific toxicity associated with exceed the limit of these important factors(1). The limitations of gentamycin to one week course of therapy is to avoid the nephrotoxicity and others adverse effects. The glomerulopathies or tubulopathies of gentamycin mediated by change the negativity of basement membrane(2). The mechanisms of gentamycin toxicity may be attributed to change the filtration or reabsorption process by changing the pores diameter or

blocking the transporters capacities that associated with changing of oncotic or hydrostatic pressure that ended with reduction of glomerular filtration rate and deterioration in renal functions(3, 4). The reduction of blood flow to the afferent arterioles was leading to reduce the hydrostatic pressure beside reducing sufficient requirement from oxygenated blood that create ischemic environment that lead to bad progression of toxicity(5).

Sildenafil (SLD) has phosphodiesterase inhibitor causing smooth muscles of blood vessels to relax and increased blood flow to certain area. Researchers view deal with searching a drug that is providing an anti-inflammatory, antioxidant, and increasing blood flow in order to reverse the gentamycin toxicity that explain the cause of selection of sildenafil for this study to investigate its effect the gentamycin toxicity(6).

Material and Methods

Animals and experimental design

Male Sprague Dawley rats with weight (200–250 g) were kept 10 days in cages for acclimatization at suitable conditions and were fed with regular water and food. Thirty-two healthy domestic male rats were randomly allocated into four groups, each group of eight animals and treated according to study protocols for 10 consecutive days as following: The first (control) group received isotonic saline (2mL/kg/day i.p.). Second (positive control) group were received only sildenafil (5 mg/kg/day orally p.o) (7).Third (induction) group received gentamycin (100 mg/kg/day i.p.) (8). Fourth (treatment) group received sildenafil (5mg/kg/day p.o.) followed by gentamycin (100 mg/kg/day i.p.) (7). The animals were sacrificed after anesthetized at day 11^{th}. Serum were prepared after centrifugation of blood samples that drawing from heart of all animal to assess serum levels of creatinine, urea, albumin, total protein .

Determination of serum creatinine level

The concentrations of serum creatinine were done according to Jaffe reaction by using a specific kit. The principle be governed by the reaction of creatinine with picric acid under alkaline conditions to form a recolored product which is directly proportional to creatinine concentration that can be measured at 500 nm. The red color intensity was expressed in (mg/ml) (9).

Determination of serum urea level

Levels of serum urea were determined using urease-modified Berthelot reaction. the ammonium ion reacts with the hydrochlorides and salicylate to form a green colored indophenol (2, 2 dicarboxylic indophenol), in an alkaline medium, which can be measured by auto-analyzer spectrophotometer at 580 nm and levels of urea was expressed in (mg/ml) (10).

Determination of serum albumin level

Principle of the method of determining the albumin levels in the presence of bromcresol green at a slightly acid pH produces a color change from yellow-green to green-blue as an indicator. The intensity of the color formed to the albumin concentration is proportionally relationship in the sample as expressed in (g/dl)(11).

Determination of serum total protein

Serum total protein was determined by using Biuret colorimetric method. Proteins give an intensive violet-blue complex with copper salts in an alkaline medium represent the principle of assay. An antioxidant is used for example iodide. The intensity of the color formed to the total protein concentration in the sample is a direct proportion which was expressed in (g/dl)(12) .

Histopathological examination of renal tissues

Formalaldehyde (10%) was used for sample fixation of the right kidneys, which were then immersed in paraffin wax, cut into 5 μm sections, then stained by hematoxylin and eosin (H&E), then the examination done with a microscope at 125x and 500× magnification to detect the renal injury for damaged glomeruli, tubular necrosis, tubular dilation, cast formation, and congestion(13, 14) .

Statistical analysis

Data were expressed as means ± S.E.M. using SPSS V20. The parametric test, one-way analysis of variance (ANOVA), followed by post hoc Tukey test were selected to compare the difference of mean at $P \leq 0.05$.

Results

The study showed that administration of gentamycin to rats (induction group) induced nephrotoxicity indicated by producing a significant elevation in serum creatinine, urea, and KIM-1levels. Whereas, it produce a significant reduction in serum total protein and albumin levels when compared to the control and sildenafil groups, respectively (Table 1).

Sildenafil when used concomitantly with gentamicin (treatment group) exerts a nephroprotective effect , evidenced by a significant decrease in serum creatinine, urea, and KIM-1levels, with a significant increase in serum total protein and albumin levels when compared with the induction groups, besides there are no changes in serum biomarkers excluding serum KIM-1 level when compared to the control and sildenafil groups, respectively (Table 1).

Table 1: Effect of sildenafil on serum biomarkers.

Groups	Serum Mean ± S.E.M				
	Creatinine (mg/dl)	Urea (mg/ml)	Total protein (g/dl)	Albumin (g/dl)	KIM -1 (g/dl)
Control	0.91±0.02	34.097±0.14	7.055±0.25	4.83±0.29	30.55±2.53
Sild. (positive control group)	0.90±0.01	32.097±0.13	7.952±0.16	4.79±0.30	31.55±1.55
GTN (Induction group)	2.643±0.15 a,b	118.98±0.18 a,b	4.100±0.13 a,b	2.357±0.04 a,b	140.1±0.32 a,b
SLD. + GTN (Treatment group)	1.04 ±0.03 c	43.346± 1.33 c	6.598±0.10 c	3.758±0.25 c	54.43±0.93 a,b,c

a = significant change when being compared to control group; b = significant change when being compared to that of Sild. group ; c = significant change when being compared to GNT group.

The histopathological examination showed no changes in the kidney tissues of control group (as shown in figures 1, 2 and 3) . From figures 4, 5 and 6 , the induction group showed an intensive inflammation in the periglomerular area and noticeable rupture of Bowman's capsule with interstitial hemorrhage. Furthermore, it exhibiting marked vacuolation of the renal tubular epithelium accompanied with renal

vascular congestion and interstitial edema. Also, it exhibited an obvious sloughing of the renal tubular epithelium and hyaline cast deposited in the lumen of renal tubular. While sildenafil (treatment) group showed a decrease in interstitial inflammation, besides a marked absence of hyaline cast in the renal tubular lumen (as shown in figures 7 and 8).

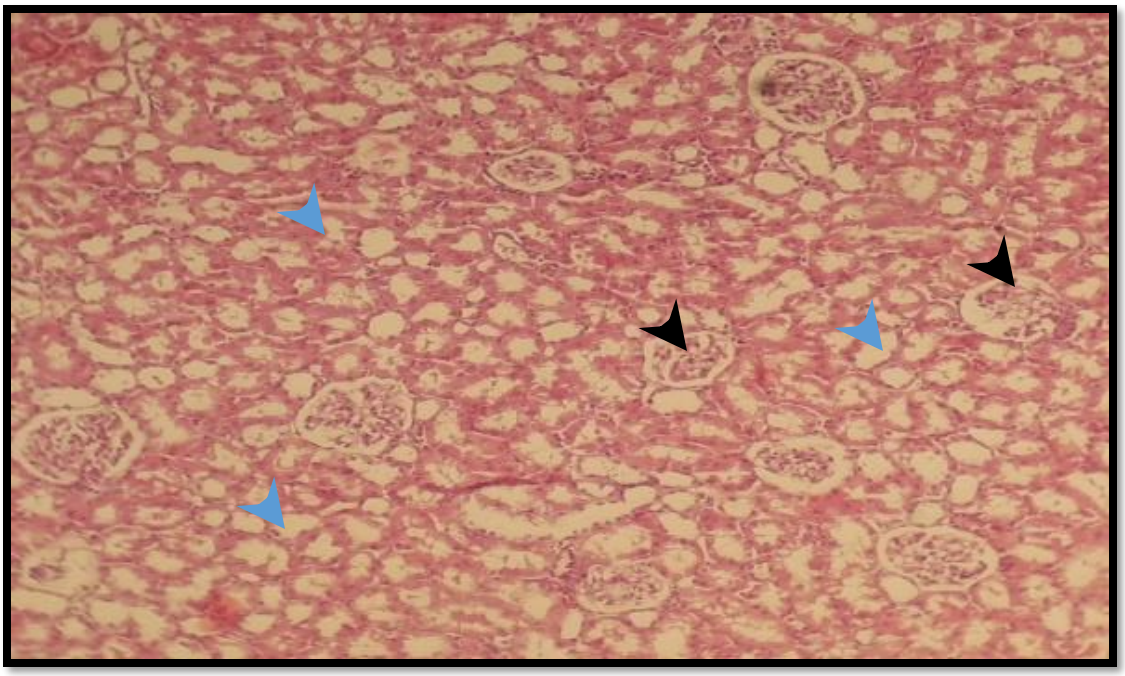


Figure (1): Section of kidney of control group showed a normal glomeruli (black arrow head), normal renal tubules (blue arrow head) H&E 125X

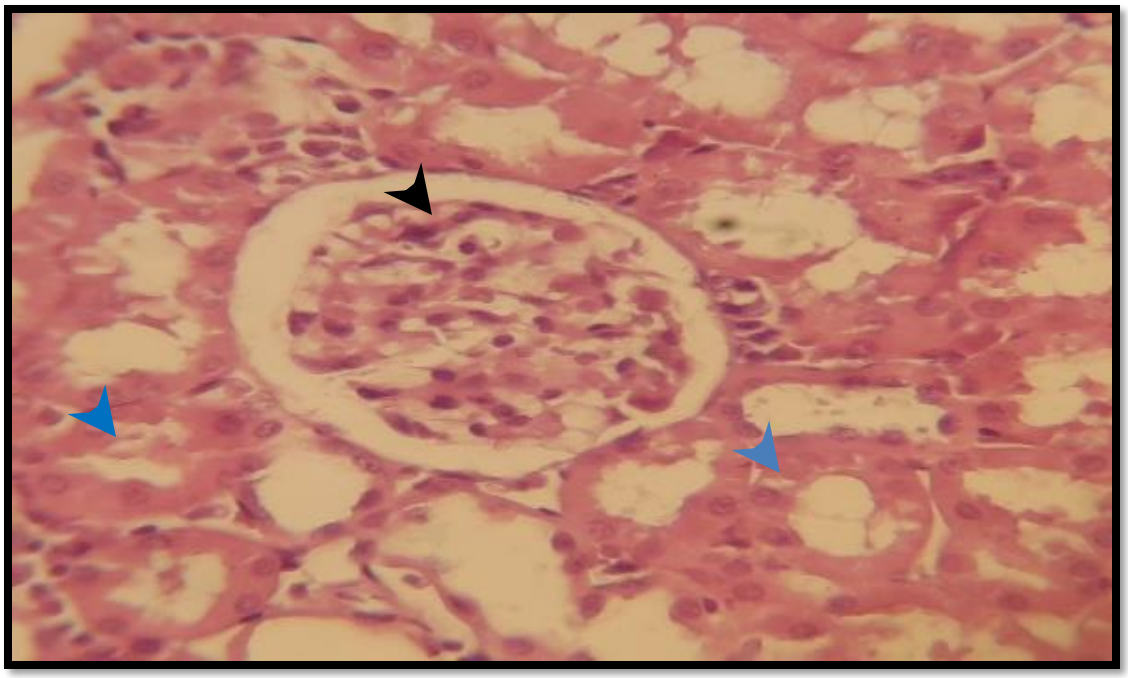


Figure (2): Section of kidney of control group showed normal glomeruli (black arrow head), normal renal tubules (blue arrow head) H&E 500X

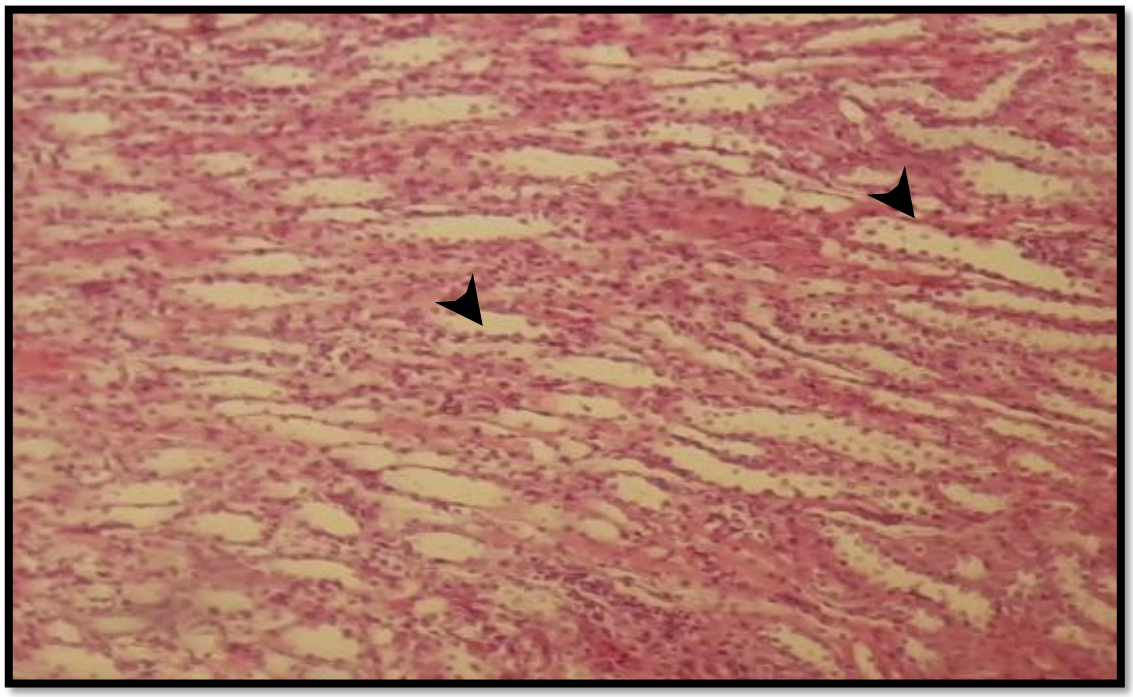


Figure (3) : Section of kidney of control group showed normal renal tubules in the medullary region (black arrow head). H&E 125X

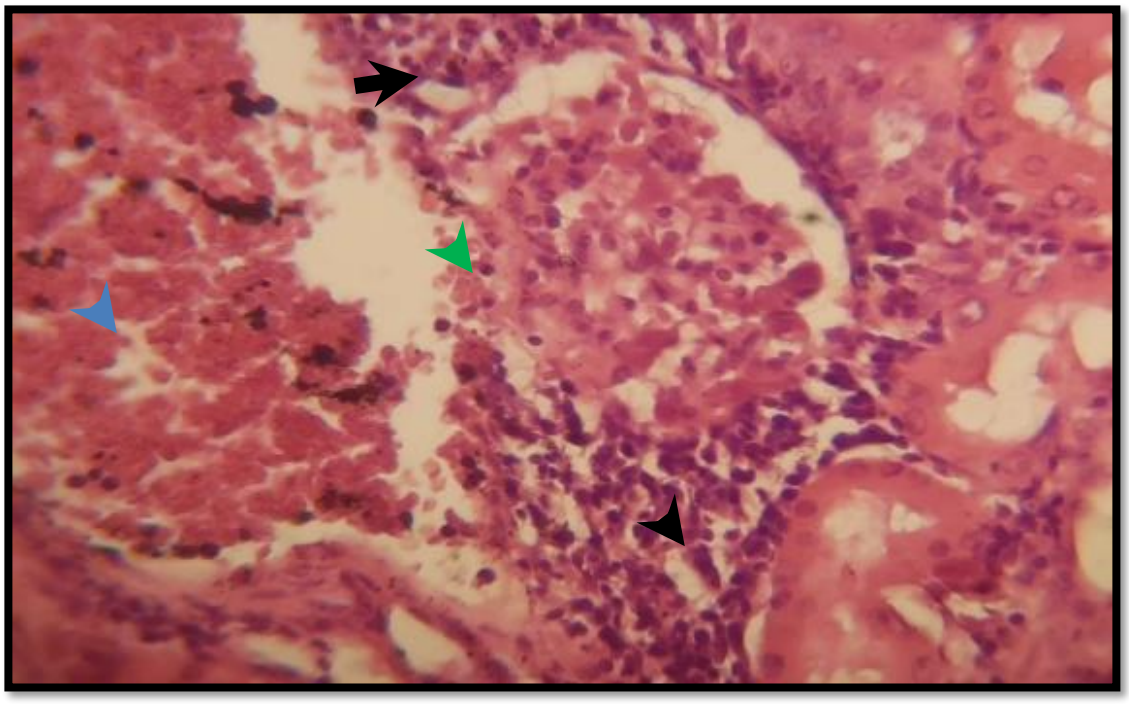


Figure (4): Section of kidney of induction group showed intensive inflammation in the periglomerular area (black arrow head), rupture of bowman's capsule (green arrow head), interstitial hemorrhage (blue arrow head) H&E 500X

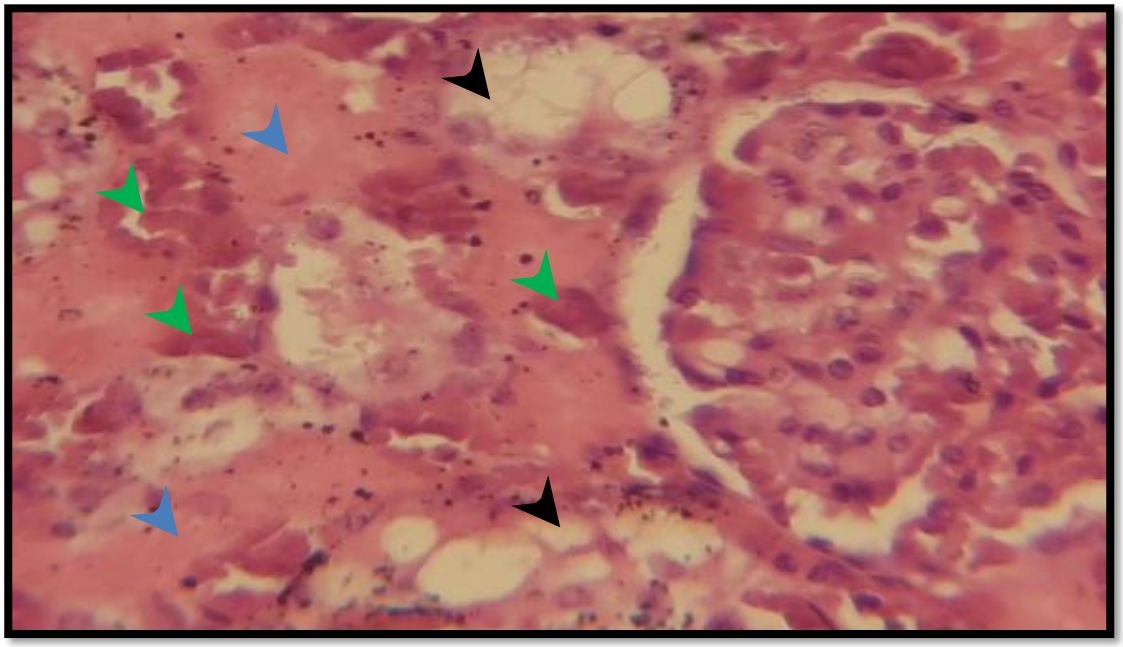


Figure (5): Section of kidney of induction group shows marked vacuolation of the renal tubular epithelium (black arrow head), renal vascular congestion (green arrow head), interstitial edema (blue arrow head) H&E 500X

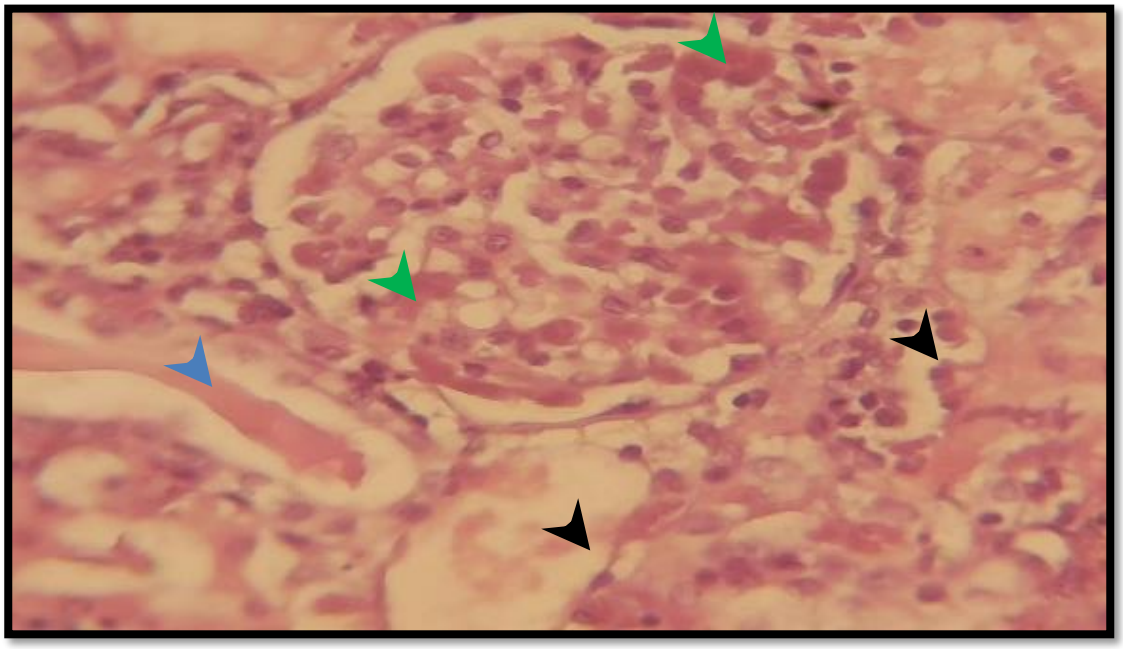


Figure (6): Section of kidney of induction group showed marked sloughing of the renal tubular epithelium (black arrow head), glomerular congestion (green arrow head), hyaline cast in the renal tubular lumen (blue arrow head H&E 500X

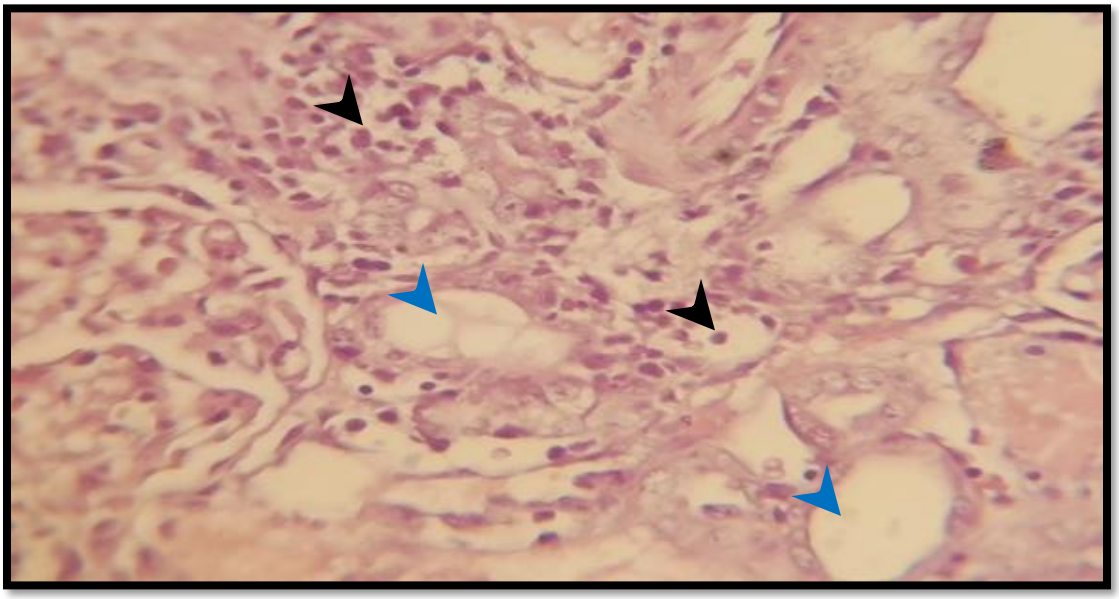
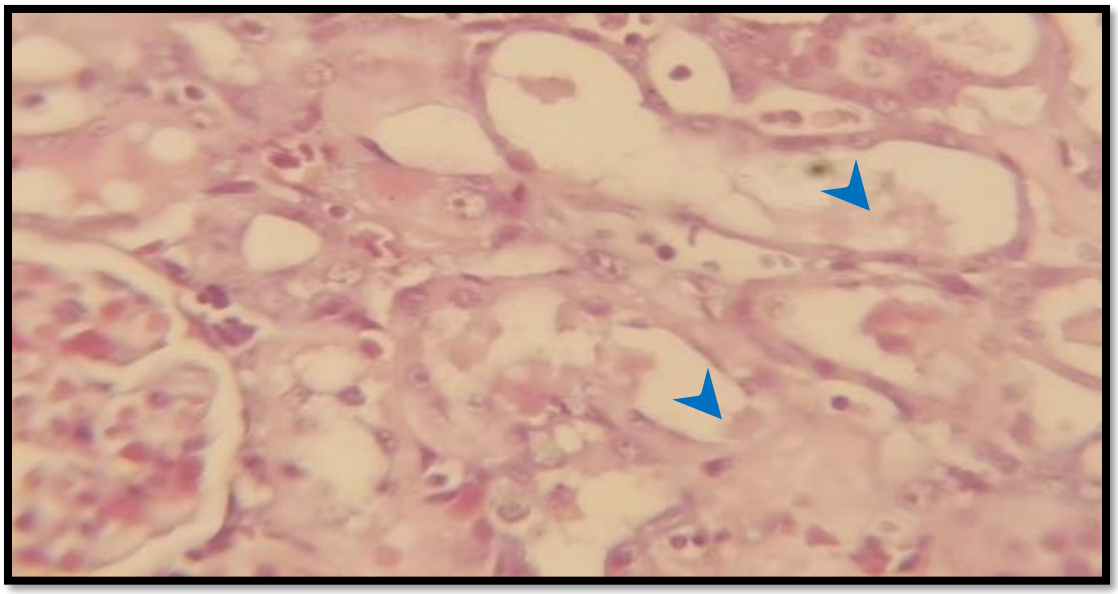


Figure (7): Section of kidney of Sildenafil (treatment) group mild inflammatory infiltration in the interstitium (black arrow head), normal renal tubules (blue arrow head) H&E 500X



Figure(8): Section of kidney of sildenafil treatment) group, mild hyaline material renal tubular lumen (blue arrow head) H&E 500X

Discussion

Functional and morphological impact of aminoglycosides nephrotoxicity is well established. The common member of aminoglycosides is a gentamycin which has proved to possess serious nephrotoxic side effects that may end in acute renal failure(15,16). In the present study, gentamicin toxic dose causes significant increase in creatinine levels besides urea levels. Gentamycin toxicity may be mediated by induction of apoptosis and necrosis(16), while another study showed it causing vasoconstriction of afferent arterioles through increasing the endothelin-1 and trigger inflammation and oxidative reactions cascades(17). All these events, ended with reduction of GFR and elevation of urea of creatinine level while increasing the permeability of glomeruli to proteins especially albumin to be excreted in urine. Another study showed the reduction of serum albumin resulted from reduction of hepatic biosynthesis in addition to their extensive excretion (18).

Sildenafil ameliorated gentamicin-induced nephrotoxicity that approved in the current study through significant reduction in creatinine levels and improve renal function that may be attributed to inhibitory effect on lipid peroxidation and oxidative stress sequels. In another study, sildenafil showed a suppressing effect on inducible nitric oxide synthase iNOS expression and limiting the glomerular injury and subsequent glomerulosclerosis (19, 20). Sildenafil had a potent and highly selective PDE-5 inhibit or beyond the restoration of GFR and improving the serum levels of both urea and creatinine by enhancing NO induced cGMP formation and accumulation(21). Other studies displayed that sildenafil is considerably enhancing the renal blood flow by stimulating intra-cellular cGMP in ischemic acute renal failure in rats. The results of this study is compatible with other studies(22, 23). Sildenafil reduces the level of KIM-1 that is expressed in the proximal tubule in the ischemic rat kidney to be markedly up-regulated (24). In addition , the histopathological changes reflected an improving effect of sildenafil by reducing the inflammation pictures and reducing the hyaline casts. This results were compatible with other studies (25-26). This effect may be due basic mechanism of sildenafil PDE-I besides anti-inflammatory effects as shown with other member of PDE-Is (27, 28).

Conclusion

Sildenafil protected against nephrotoxicity-linked renal morphological damage and renal function disturbances, by decreasing renal oxidative stress and inflammation.

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Conflict of interest

The authors declare that there is not conflict of interest concerning the publication of this manuscript.

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