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Evaluation of the effect of administration of Tadalafil on Gentamicin-induced nephrotoxicity in rats

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Abstract:

Background: Gentamicin (GNT) is a highly effective aminoglycoside antibiotic that is commonly used to treat life-threatening bacterial infections. **Aim:** The aim of the current study is to determine whether tadalafil can protect against GNT-induced nephrotoxicity. **Methods:** Twenty-four male Albino rats were used in the study, which were randomly divided into four groups, six animals each. Control untreated group received distilled water (5ml/kg, P.O) for 12 days and on days 6-12, they received (5ml/kg,i.p) normal saline daily, one hour after oral administration of distilled water. Tadalafil group received tadalafil (5mg/kg, P.O) for 12 days and on days 6-12, they received (5ml/kg,i.p) of normal saline daily, one hour after oral administration of tadalafil. Gentamicin group received distilled water (5ml/kg, P.O) and on days 6-12, they received gentamicin (١٠٠mg/kg,i.p) one hour after oral administration of distilled water. Gentamicin+Tadalafil group received tadalafil (5mg/kg, P.O) for 12 days and on days 6-12, they received gentamicin (100mg/kg,i.p) one hour after oral administration of tadalafil. Body weight and kidney weight were investigated. Urine volume as well as urinary albumin, creatinine, creatinine clearance and albumin /creatinine ratio **(ACR)** were evaluated. Besides, serum levels of urea and creatinine were measured as kidney function parameters. Renal tissues oxidative stress markers as malondialdehyde (MDA) and the antioxidant enzymes as superoxide dismutase (SOD) and catalase (CAT) activities. Renal cortex nitric oxide (NO) and kidney injury molecule-1 (KIM-1) were evaluated. Histological analysis and immunohistochemical expression of endothelial nitric oxide synthase (eNOS) and inducible nitric oxide synthase (iNOS) were

performed. The results of the present study showed that GNT decreased creatinine clearance and increased the serum levels of renal function parameters. GNT caused a significant increase in renal cortex MDA, NO and urine levels of KIM-1, while it decreased CAT and SOD activities. Gentamicin administration resulted in increased immunohistochemical expression of iNOS enzyme while decreasing eNOS expression. Renal corpuscles and tubules also showed histological and ultrastructural changes. Pretreatment with tadalafil, on the other hand, reversed the effects of GNT administration. **In conclusion**, findings of the present study indicate that tadalafil reduces GNT-induced kidney damage by inhibition of inflammation, oxidative stress, and apoptosis.

Keywords: Gentamicin- Aminoglycoside- Nephrotoxicity- Tadalafil- iNOS-eNOS- KIM-1

1. Introduction:

The aminoglycoside antibiotic GNT is used to treat many types of bacterial infections, particularly those caused by gram-negative bacteria. With a concentration-dependent antibacterial activity and post-antibiotic effect, it is highly effective against gram negative bacilli (1).

Due to its chemical stability, quick bactericidal action, interaction with ß lactam antibiotics, little resistance frequency, and virtually lower cost, GNT is a charismatic antibiotic. Unfortunately, these benefits are mostly linked to complications of nephrotoxicity and ototoxicity (2).

Nephrotoxicity is the major implied side effect of GNT therapeutic dosages. It has been reported that some symptoms of nephrotoxicity are present in up to 30 percent of patients treated with gentamicin for more than 7 days (3). Renal damage is often expressed by an increase in creatinine and urea nitrogen serum levels (4).

Tadalafil that has a long-lasting impact, inhibits phosphodiesterase enzyme-5 (PDE5), which hydrolyzes cyclic guanosine monophosphate (cGMP). Therefore, the PDE5 inhibition with tadalafil increases both cGMP and nitric oxide (NO) (5).

For the treatment of renal dysfunction, PDE5 inhibitors like tadalafil are used. In models of diabetic nephropathy, renal ischemia-reperfusion injury and chronic kidney disease, regular treatment with PDE5 inhibitors could attenuate kidney injury and blood pressure elevation. The NO/cGMP signaling inhibition in the kidney can also induce renal dysfunction. PDE5 inhibitors facilitate relaxation of the vascular smooth muscle and, subsequently, contribute to a marked reduction of BP. They may also be novel, effective

therapies for renal dysfunction, as they increase the level of cGMP (6). As cGMP plays an important role in the NO-mediated inhibition of leukocyte adhesion, both increased NO production and increased cGMP levels may contribute to the anti-inflammatory effect (7).

PDE 5 inhibitors (e.g., tadalafil) have been reported to cause relaxation of smooth muscle cells in the neck of the urethra, prostate and bladder. Tadalafil has recently gained popularity for the control of lower urinary tract symptoms secondary to benign prostatic hyperplasia (8).

The present study aimed to determine the ability of tadalafil to protect rat kidney from gentamicin-induced nephrotoxicity and to demonstrate the possible mechanisms of actions.

2. Maerials and Methods:

A-Drugs and chemicals

- **Gentamicin:** (Gentamicin Sulphate ampoule 80mg/2ml, Memphis Co. for Pharmaceutical&Chemical Industries, Cairo, Egypt).
- **Tadalafil: (**Cialis 20mg tablets, Lilly del Caribe Inc., Puerto Rico Industrial Park, Carolina - Puerto Rico).
- **Saline (0.9%NaCl): (**Isotonic saline, Al Mottahedoon Pharma for Pharmaceutical Medical Production and Cosmetics, 10th of Ramadan City, Egypt).
- **Distilled water.**
- **Materials required for histopathological evaluation:** Hematoxylin and Eosin (H&E).
- **Materials required for Immunohistochemical evaluation and biochemical parameters:**

- Antibody to inducible nitric oxide synthase (iNOS), Antibody to endothelial nitric oxide synthase (eNOS), kits for urea, creatinine, albumin, super oxide dismutase (SOD), catalase, malondialdehyde and nitrite.

All previous kits were purchased from Biodiagnostic Co., Badr City, (Egypt).

- Rat kidney injury molecule-1, KIM-1 ELISA Kit was purchased from My BioSource, Vavcouver, (British Columbia).

B-Animals:

Twenty- four adult male Albino rats obtained from animal house of Faculty of Pharmacy, Nahda University in Beni Suef weighting 150-200 gm were used after one week for proper acclimatization to the standard housing conditions (25 ± 2 ^{o}C temperature and 12 h light/dark cycle) and were supplied with standard rodent chow and tap water ad libitum (9).

The animals were handled according to the guidelines of the local ethical committee, Faculty of Medicine, Beni-Suef University, which comply with the international guidelines for the use and care of laboratory animals. The ethics committee approval for animal was obtained (# 020-97).

Experimental Design:

The twenty-four male Albino rats were randomly divided into four groups (six rats each):

Group 1 (Control untreated): Rats in this group were given distilled water (5ml/kg) orally for 12 days and on days 6-12, they received an i.p. injection of normal saline daily (5ml/kg) one hour after oral administration of distilled water.

Group 2 (Tadalafil group): Rats were given tadalafil orally (5mg/kg) **(10)** for 12 days and on days 6-12, they received an i.p. injection of normal saline daily (5ml/kg) one hour after oral administration of tadalafil. The tablets were crushed and freshly suspended in distilled water and administered orally by a metallic tube.

Group 3 (Gentamicin group): Rats were given distilled water (5ml/kg) orally and on days 6-12, they received an i.p. injection of gentamicin 100mg/kg one hour after oral administration of distilled water (11).

Group4 (Gentamicin+Tadalafil group): Rats were given tadalafil orally (5mg/kg) for 12 days and on days 6-12, they received an i.p. injection of gentamicin 100mg/kg one hour after oral administration of tadalafil.

The rats were sacrificed 24 h following the last gentamicin injection, blood samples were collected from venous plexus deep to medial canthus of the palpebral fissure using 21 G needles mounted upon a 5ml syringe. Each blood sample obtained from rats of each group was collected into a well labeled 10ml capacity plain sample, and serum was separated by centrifugation at 2500 xg for 20 minutes. Serum was used for estimation of urea and creatinine levels. For histopathological and immunohistochemical study, kidneys were removed, washed and dried. A longitudinal section from right and left kidneys from each animal was excised. The renal cortex of the kidneys (renal cortex appears lighter in color compared to the rest of the kidney) was stored at –80 º C and subsequently homogenized in ice-cold phosphate buffer for biochemical analysis of renal cortex catalase, SOD, MDA, NO.

Parameters to be investigated:

1. Body weight, kidney weight, percent change of body weight, and kidney weight/body weight ratio.
2. Serum levels of urea, creatinine.

3. Rat Kidney injury molecule 1.
4. Renal catalase, superoxide dismutase activity and renal cortex malondialdehyde content.
5. Renal cortex nitric oxide (NO) content.
6. Urine volume (*animals were accommodated in metabolic cage for 24 hours for urine collection),* urinary albumin, urinary creatinine, and urinary albumin /creatinine ratio (ACR).

   Creatinine clearance (ml/min) were estimated with formula:

- Clcr=urine creatinine (mg/dL) x urine flow (mL/min) /Serum creatinine (mg/dL) (12).

Statistical analysis:

SPSS v. 25 (Statistical Package for Social Science) for Windows was used to analyse the results.

Description of variables was presented as follows:

- Description of quantitative variables was in the form of mean, standard deviation (SD) for normally distributed variables.
- The normality of all variables was investigated. Both variables appear to be distributed normally.
- One-way ANOVA test was used to detect the difference between the four groups regarding the scale variables and Tukey post hoc high significant degree was conducted for multiple comparisons between groups

3. Results:

Effect of tadalafil on body weight and percent change of body weight of gentamicin-induced nephrotoxicity:

The results of the present study showed that there were no statistically significant differences between the four groups regarding the percent change in body weight before and after treatment, or the body weight both before and after treatment but, there was a significant increase in body weight after the treatment in each group (**Table 1)**.

Table (1): Effect of treatment on the body weight and percent change of body weight in each group:

	Body weight (BW)		**% change of body weight**
	Before (mean ± SD)	**After (mean ± SD)**	
Control	170.8±16.8	208.3±17.5*	-22.2±6.2
Tadalafil	179.2±14.6	220.8±27.5*	-23.1±10.1
Gentamicin	171.7±8.2	203.3±16.3*	-18.6±9.7
Gentamicin+Tadalafil	161.7±5.2	196.7±10.8*	-21.7±7.5

* Significant at P-value <0.05 compared to before treatment in each corresponding group.
(-values) means it is a percent of increase as the post value is larger than the pre value.

Effect of tadalafil on kidney weight and kidney weight/body weight ratio of gentamicin-induced nephrotoxicity:

The results of the present study showed that there were no statistically significant differences between the four groups regarding kidney weight after treatment. Gentamicin does not cause a significant difference in kidney weight or kidney weight/body weight ratio (relative kidney weight) **(Table 2).**

Table (2): Comparison between groups regarding the kidney weight and kidney weight/body weight ratio (relative kidney weight) after treatment:

	Kidney weight after treatment (gm)	**Kidney weight/body weight ratio after treatment (%)**
Control	1.7±0.1	0.7±0.04
Tadalafil	2±0.6	0.9±0.20
Gentamicin	2±0.3	1±0.20
Gentamicin+Tadalafil	1.9±0.11	0.98±0.10

Effect of tadalafil on the serum urea and serum creatinine levels of gentamicin-induced nephrotoxicity:

The results of the present study showed that there were significant differences between the four groups regarding the serum creatinine and urea levels (P-value <0.001). A significant increase in the serum creatinine and urea levels was observed in the gentamicin group compared to the control group. This increase in the serum creatinine and urea levels was significantly alleviated in the gentamicin +tadalafil group (**Table 3)**.

Table (3): Comparison between the four groups regarding the serum urea level and the serum creatinine level:

	Serum urea (mg/dl)	**serum creatinine (mg/dl)**
Control	34.9±11.1	0.18±0.079
Tadalafil	37.9±15.60	0.19±0.090
Gentamicin	90.5±18.1*	1.02±0.210*
Gentamicin+Tadalafil	51.4±13.9#	0.42±0.110#

Data are expressed as Mean±SD of 6 rats.

*Statistically different from Control group at P-value < 0.05.

#Statistically different from Gentamicin group at P-value < 0.05.

Effect of tadalafil on the urine albumin level, urine creatinine, creatinine clearance, albumin/creatinine ratio and 24hour urine volume of gentamicin-induced nephrotoxicity:

The results of the present study showed that there were significant differences between the four groups regarding the urine albumin level, creatinine clearance, urine creatinine and albumin/creatinine ratio (P-value <0.001). A significant increase in the urine albumin level and albumin/creatinine ratio was observed in the gentamicin group compared to the control group. This increase in the urine albumin level and albumin/creatinine ratio was significantly alleviated in the gentamicin+ tadalafil group.

As regarding urine creatinine level, a significant decrease in the urine creatinine level was observed in the gentamicin group compared to the control group. This decrease in the urine creatinine level was significantly alleviated in the gentamicin+tadalafil group.

As regarding creatinine clearance, a significant decrease in the creatinine clearance level was observed in the gentamicin group compared to the control group. This decrease in the creatinine clearance was significantly alleviated in the gentamicin+tadalafil group.

As regarding 24hour urine volume after treatment, the results of the present study showed that there was no significant difference between the four groups. Gentamicin does not cause a significant difference in the 24hour urine volume in the gentamicin group **(Table 4)**.

Table (4): Comparison between the four groups regarding the urine albumin, urine creatinine, albumin/creatinine ratio and 24hour urine volume levels after treatment:

	Urine albumin (mg/dl)	**Creatinine clearance (ml/min)**	**Urine creatinine (g/dl)**	**Albumin/Creatinine ratio after treatment (mg/gm)**	**24hour urine volume after treatment (ml)**
Control	402.92±54.05	222.85±112.89	0.043±0.004	9.5±1.3	11.6±60
Tadalafil	401.78±32.83	290.73±40.75	0.041±0.004	9.9±0.9	19.4±7.9
Gentamicin	689.75±30.25*	16.75±15.45*	0.015±0.004*	49.8±13.7*	17.5±15.9
Gentamicin+ Tadalafil	489.95±47.93#	124.17±61.65#*	0.033±0.003#	15.2±1.8#	19.5±6.8

Data are expressed as mean±SD of 6 rats.

*Statistically different from Control group at P-value ≤ 0.05.

#Statistically different from Gentamicin group at P-value ≤ 0.05.

Effect of tadalafil on the renal catalase, tissue superoxide dismutase and tissue malondialdehyde contents of gentamicin-induced nephrotoxicity:

The results of the present study showed that there were significant differences between the four groups regarding the renal catalase, renal superoxide dismutase activity. A significant decrease in the renal catalase and tissue superoxide dismutase activity was observed in the gentamicin group compared to the control group. This decrease in the renal catalase and superoxide dismutase acticity was significantly alleviated in the gentamicin+tadalafil group.

A significant increase in the renal MAD content was observed in the gentamicin group compared to the control group. This increase in the renal MAD content was significantly alleviated in the gentamicin+tadalafil group. **(Table 5).**

Table (5): Comparison between the four groups regarding the renal activity of catalase & tissue superoxide dismutase as well as content of malondialdehyde:

	tissue catalase (U/gm tissue)	**tissue superoxide dismutase (U/gm tissue)**	**Tissue malondialdehyde (nmol/gm tissue)**
Control	**118.95±7.56**	**26.41±7.20**	**38.76±9.54**
Tadalafil	**126.45±8.95**	**29.77±6.57**	**38.23±9.15**
Gentamicin	**55.4±8.36***	**9.85±1.84***	**121.58±11.27***
Gentamicin+Tadalafil	**100.6±7.72 #**	**19.78±3.43#**	**58.67±15.05#**

Data are expressed as mean±SD of 6 rats.

*Statistically different from Control group at P-value < 0.05.

#Statistically different from Gentamicin group at P-value < 0.05.

Effect of tadalafil on the tissue nitrous oxide level of gentamicin-induced nephrotoxicity:

The results of the present study showed that there were significant differences between the four groups regarding the tissue NO level (P-value <0.001). A significant increase in the tissue NO level was observed in the gentamicin group compared to the control group. This increase in the NO level was significantly alleviated in the gentamicin+tadalafil group. **(Table 6)**.

Table (6): Comparison between the four groups regarding the tissue nitric oxide content:

	tissue nitric oxide (nmol/gm tissue)
Control	**13.43±2.77**
Tadalafil	**14.57±5.18**
Gentamicin	**73.98±18.58***
Gentamicin+Tadalafil	**31.23±10.05#**

Data are expressed as mean±SD of 6 rats.

*Statistically different from Control group at P-value < 0.05.

#Statistically different from Gentamicin group at P-value < 0.05.

Effect of tadalafil on gentamicin-induced kidney injury (Urine kidney injury molecule type 1 (KIM-1) level: The results of the present study showed that there were significant differences between the four groups regarding the urine KIM-1 level (P-value <0.001). A significant increase in the urine KIM-1 level was observed in the gentamicin group compared to the control group. This increase in the the urine KIM-1 level was significantly alleviated in the gentamicin+tadalafil group **(Table 7)**

Table (7): Comparison between the four groups regarding the urine KIM-1 level:

	Urine kidney injury molecule type 1 (KIM1) (pg/ml)
Control	**9.22±1.47**
Tadalafil	**9.24±1.92**
Gentamicin	**46.23±10.53***
Gentamicin+Tadalafil	**14.45±1.68#**

Data are expressed as mean±SD of 6 rats.

*Statistically different from Control group at P-value < 0.05.

#Statistically different from Gentamicin group at P-value < 0.05.

Histopathological results

Effects of tadalafil and gentamicin on renal histopathology:

Histopathological analysis revealed that normal characteristics of renal glomeruli and cortical tubules were observed in the control and tadalafil groups **(Figures 1(A) and 1(B)) .** In comparison, gentamicin-treated rats displayed degeneration and necrobiosis in the epithelial cells lining the renal tubules **Figures 1(C).** The histopathological insult caused by gentamicin was restored by concomitant administration of tadalafil with gentamicin as it showed regular epithelial cells lining the tubules **Figure1 (D).**

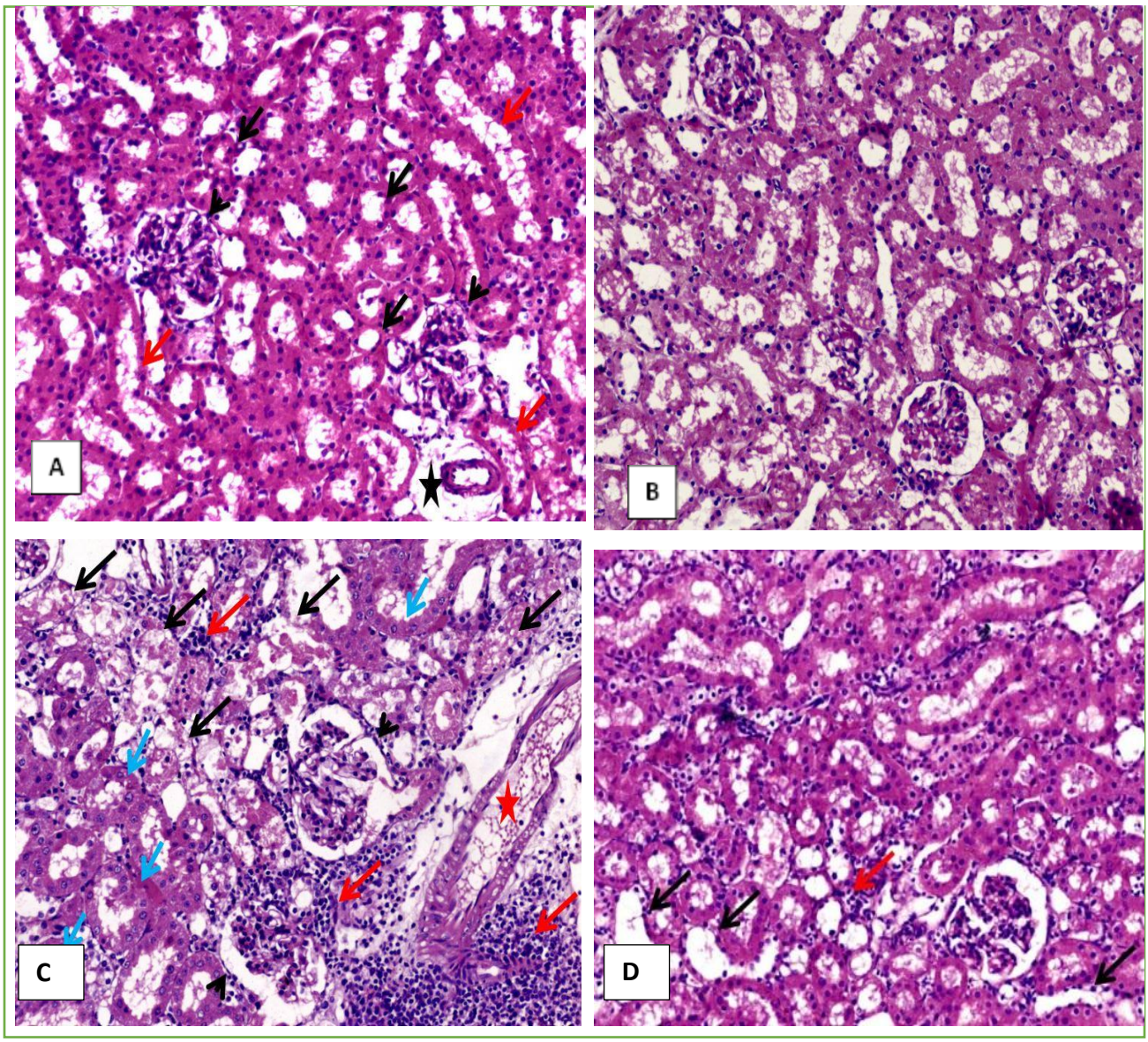

Figure (1): Light microscopy of the renal section from the gentamicin group (**A**) demonstrated glomerular atrophy noted that the surrounding diffuse acute renal tubules necrosis (black arrows) associated hydropic degeneration in few tubules (blue arrows). The interstitial tissue showed edema and moderate to severe lymphoplasmacytic cellular infiltrates (red arrows), with marked dilated congested cortical capillaries (red star) (H&E stain ×200). Tadalafil group **(B),** renal tissue demonstrated unremarkable histopatholog ical changes (H&E stain ×200). Gentamicin group **(c),** light microscopy of the renal section from the gentamicin group demonstrated glomerular atrophy

noted that the surrounding diffuse acute renal tubules necrosis (black arrows) associated hydropic degeneration in few tubules (blue arrows). The interstitial tissue showed edema and moderate to severe lymphoplasmacytic cellular infiltrates (red arrows), with marked dilated congested cortical capillaries (red star) (H&E stain ×200). Gentamicin and tadalafil group **(D),** Light microscopy of the renal section from the gentamicin and tadalafil group demonstrated predominant normal renal morphology with only occasional tubular degenerative changes (black arrows) and reduced interstitial inflammatory infiltrates (red arrow) this indicated best nephroprophylactive activity against gentamicin-induced nephrotoxicity

Immunohistochemical results:

Effects of tadalafil and gentamicin on renal inducible nitric oxide synthase (iNOS) and endothelial nitric oxide synthase (eNOS) expression:

Sections of the control rat and tadalafil group demonstrated negative iNOS immunoreaction in the tubular cells' cytoplasm **(Figures 2(A) and 2(B)).** Meanwhile, sections of gentamicin-treated rats showed a high expression of iNOS reaction in the degenerated epithelial of renal tubules **(Figures 2(C)).** In contrast, the renal cortex of rats treated with gentamicin and tadalafil showed faint iNOS expression in tubular cells' cytoplasm **(Figures 2(D)).**

Regarding eNOS immunoreaction, the control group and the tadalafil group showed intense eNOS immunoreaction in the glomerulus endothelium and faint immunoexpression in the renal tubules **(Figures 3(A) and 3(B)).**

The gentamicin-treated rats showed absent eNOS immunoreaction of the renal glomerulus and renal tubules **(Figures 3(C)).** Meanwhile, faint immunoexpression of eNOS in the renal glomerulus and renal tubules in the gentamicin-adminestered rats treated with tadalafil **(Figures 3(D))**.

Immunohistochemical staining of the rat kidney demonstrated that gentamicin administration induced a marked increase in iNOS immunoreactivity and a decrease in eNOS immunoreactivity relative to the control group. Co-

administration of tadalafil with gentamicin markedly reduced iNOS expression, whereas eNOS expression was markedly increased relative to gentamicin group.

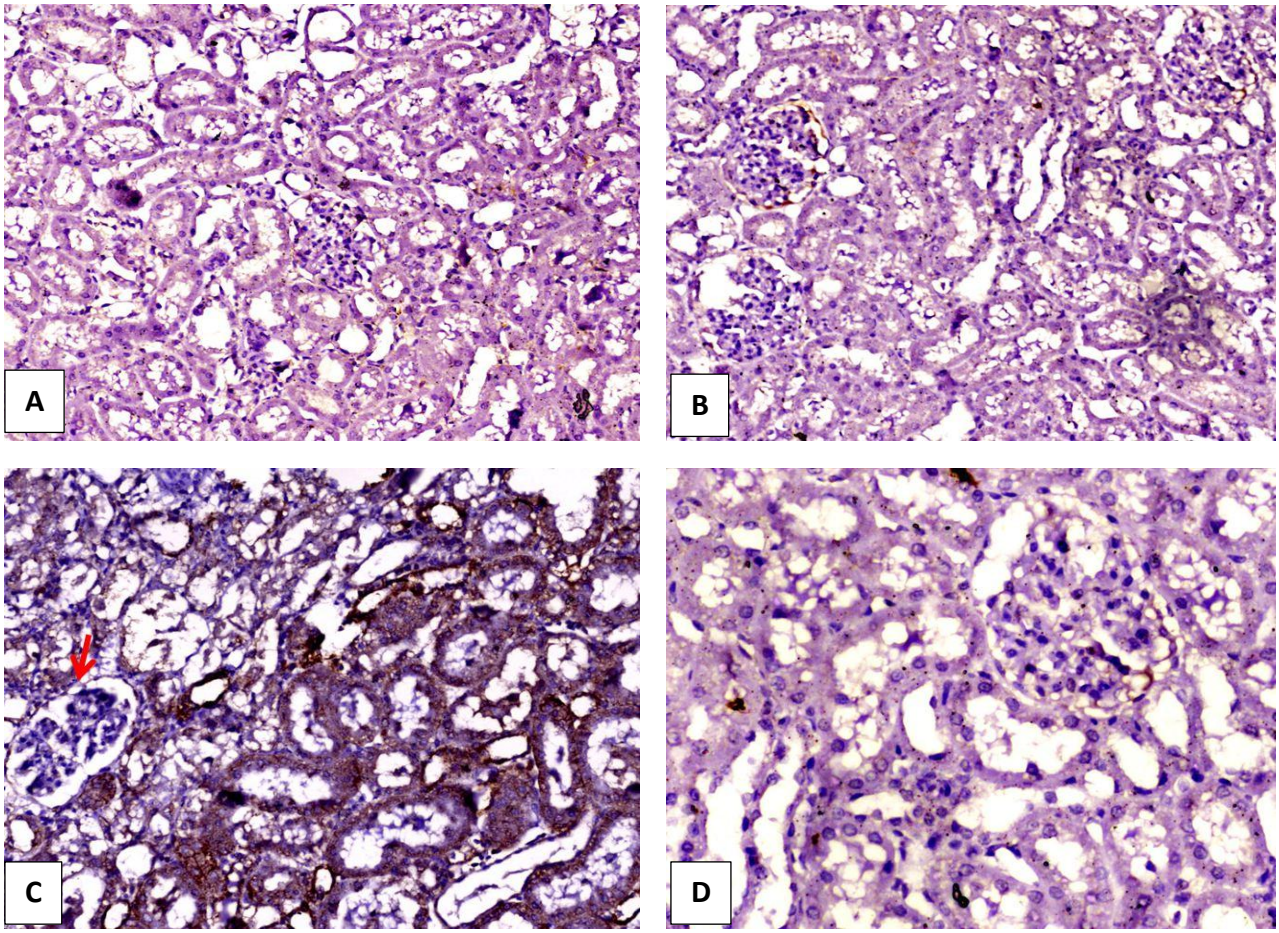

Figure (2): Light microscopy of the renal section from the control group (**A**) showed negative iNOS immunoreactivity (magnification x200). The tadalafil group (**B**) showed negative iNOS immunoreactivity (magnification x200). gentamicin group (**C**) demonstrated positive cytoplasmic iNOS immunostaining of degenerated epithelial of renal tubules with absent immunoreactivity within renal glomerulus (red arrow) (magnification x200). The gentamicin group treated by tadalafil (**D**) showed negative iNOS immunoreactivity (magnification x400).

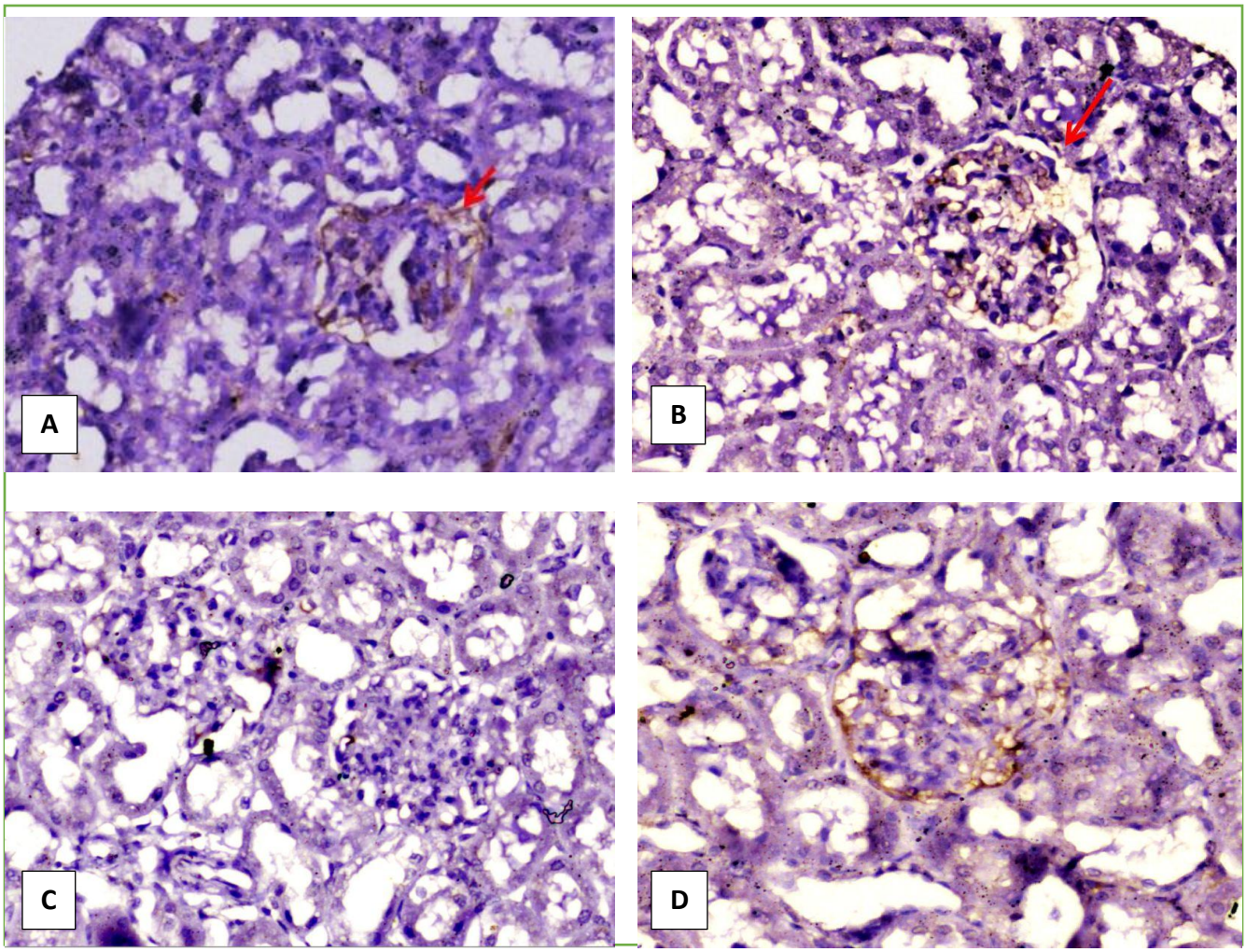

Figure (3): Light microscopy of the renal section from the control group (**A**) showed positive eNOS immunostaining in the glomerulus endothelium (arrow), (negative immunoreactivity) (magnification x400). The tadalafil group (**B**) showed positive eNOS immunostaining in the glomerulus endothelium (arrow), (negative immunoreactivity) (magnification x400). The gentamicin group (**C**) demonstrated absent eNOS immunostaining of the renal glomerulus (magnification x400). The gentamicin group treated by tadalafil (**D**) demonstrated faint immunostaining expression of eNOS in the renal glomerulus (negative immunoreactivity) (magnification x400).

4. Discussion:

Gentamicin induced nephrotoxicity was evident in the current study by a significant decrease in the creatinine clearance and urinary creatinine and a significant increase in the serum creatinine, blood urea nitrogen, urinary albumin, and urinary albumin-to-creatinine ratios due to renal impairment. This is in line with the previous studies (13,14,15,16).

In addition, gentamicin caused significant increase in concentration of urine KIM-1 as well as significant increase in renal cortex MDA and nitric oxide levels and significant decrease in SOD and catalase due to an increase in the free radical production and the depletion of antioxidant enzymes to combat oxidative stress. Gentamicin induced histological and ultrastructural changes of renal corpuscles and tubules. Gentamicin caused an increase in the expression of iNOS and a decrease in the eNOS. This is in accordance with (9,17). Despite that, GNT did not produce any significant change in the 24hour urine volume, kidney weight, body weight, kidney weight/body weight ratio.

Collectively, the findings of this study showed that GNT mediated renal injury, as evidenced by substantial renal histological damage with an increase in renal injury markers due to increased inflammatory mechanisms, oxidative stress mechanisms and NO renal tissue, as evidenced by overexpression of iNOS with eNOS suppression.

Tadalafil treatment (5 mg/kg/day for 12 days po) in gentamicin administered rats ameliorated gentamicin nephrotoxicity. Tadalafil reduced development of renal tubular necrosis and caused significant decrease in urea and creatinine levels in serum, decreased urinary albumin and albumin /creatinine ratio. Tadalafil significantly increased SOD and catalase and significantly decreased MDA, urine level of KIM-1 and renal cortex NO levels. Tadalafil increased expression of eNOS and decreased expression of iNOS in gentamicin administered rats. This is in accordance with (17,18).

Overall, the biochemical and histopathological findings of this study strongly support the nephroprophylactic impact of tadalafil in nephrotoxic rats induced by gentamicin, mediated by

antioxidant and anti-lipoperoxidant mechanisms. In the present study gentamicin injection for 1 week did not cause a significant difference in kidney weight (kw) of rats or kidney weight to body weight ratios between all groups of rats. This indicates insignificant inflammation of the tissue and edema during this period (19). This is in line with Hejazi et al. (2018) (20) who reported that there was no significant difference in mean weight of the kidney between groups of control and gentamicin breastfed newborns where lactating mothers were injected with gentamicin at (200 mg/kg i.p) every other day consecutively from the first day to the end of the period of lactation (nearly 21 days), and gentamicin was transferred to newborn through lactation. In the present study there is no statistically significant differences between the four groups of rats regarding the body weight before and after treatment. Ghaznavi et al. (2016) (17) also reported that body weight was not affected in any of the animal groups (rats were injected by gentamicin sulfate (100 mg/kg/day i.p) for 8 consecutive days and this is in accordance with the present study.

In the current study reduced creatinine clearance in gentamicin group was observed. This reflects reduced glomerular filtration rate. This is in line with (Jaikumkao et al., 2016) (21). Elevated serum creatinine concentration and blood urea concentration in gentamicin group compared to normal control rats may be an index of renal dysfunction since the kidney is cleared of urea and creatinine. Therefore, serum creatinine and blood urea elevations are an indication of renal dysfunction (22). The present findings showed that in gentamicin-treated animals, the urinary albumin/creatinine ratio (A/C ratio) is significantly higher. In renal proximal tubular cells, gentamicin accumulates, which greatly impairs the processes of reabsorption and potentially contributes to tubular obstruction with a resulting decrease in GFR and accumulation of metabolic waste products, including urea and creatinine. The low level of albumin is usually filtered through the glomeruli and is reabsorbed mostly through the proximal tubules. Increased excretion of urinary albumin is potentially related to impairment in the tubular reabsorption because of gentamicin toxicity. The induction of oxidative stress is a

significant contributing factor to the gentamicin-induced decrease in glomerular filtration rate. (24,16). The renal outcomes of patients with AKI are anuric, oliguric, and nonoliguric AKI. The urine volume is relatively normal in nonoliguric phase of AKI. Post-renal (obstructive stone nephropathy, malignancy etc), nephrotoxic drugs (as aminoglycoside antibiotics), and hypovolemia are the main causes of non-oliguric AKI other than sepsis. In patients with nonoliguric acute renal failure, these individuals appear to have less kidney damage, less morbidity and mortality and better survival rates, and so on. Diagnostic urinary indexes indicate less insult to renal function (25,26).

Other results indicated that administration of gentamicin (100 mg/kg i.p for 7 days) results in significant increase in urine volume in gentamicin treated rats (polyuric phase of AKI) (23).

In the present study, there was no signficant increase in urine volume per 24hour in gentamicin treated rats as there was no significance difference between all groups. Flow of urine was constant, indicating the occurrence of non-oliguric acute renal failure due to nephrotoxic origin.

In the current study, gentamicin administration induced nephrotoxicity. Signs of oxidative stress have been observed by significant lipid peroxide (MDA) elevation with a significant decrease in catalase and SOD in gentamicin group compared to the control group.

The decreased antioxidant activity in the nephrotoxicity caused by gentamicin may be explained by an increase in free radical production and the depletion of antioxidant enzymes to combat oxidative stress. Oxygen free radicals are essential mediators of nephrotoxicity mediated by gentamicin. Some authors have stated that gentamicin can induce free radical production by releasing iron from renal cortical mitochondria to form iron-gentamicin complexes by iron chelation, which can be the initial step in the development of gentamicin-induced ROS. The rise in the content of MDA and the low activity of antioxidant enzymes as SOD and catalase can support this viewpoint. The inactivation of these antioxidant enzymes can result from increased ROS output, which may

worsen oxidative harm (17). Dungca. (2016) (27) also have reported that administration of gentamicin (80 mg/kg/day, subcutaneous injection for seven days) increases MDA level and decreases activities of SOD and catalase by inducing oxidative stress.

The present study demonestrated increased urine KIM-1 in gentamicin-treated rats. This in line with previous reports that found elevated expression of renal KIM-1 mRNA levels of rats treated with gentamicin that was adminestred by subcutaneous injection of 200 mg/kg twice per day for 2 consecutive days to stimulate renal injury (28).

Oxidative stress has been associated with NO development by a wide body of evidence. Therefore, as indicated by renal tissue contents of nitric oxide end products (NOx), nitrite/nitrate, we investigated renal nitric oxide production. Gentamicin injection has been shown to dramatically increase renal NOx levels in gentamicin group relative to control animals. This is in line with (Abdelrahman, 2018) (29).

Unbalanced or increased ROS development and oxidative stress mediate the inflammation caused by gentamicin. Superoxide anions and hydrogen peroxides are the main mediators for many inflammatory pathways as increase iNOS leading to increased output of NO (31). In addition, iNOS-dependent eNOS inhibition further deteriorates endothelial function, forming a triangle between NO, ROS, and oxygen in the pathophysiology of oxidative stress and AKI-1 (32), and this is in accordance with the present immunohistological results. In the present study, gentamicin-induced nephrotoxicity significantly increased renal upregulation of the expression of iNOS enzymes and decreased eNOS expression. This effect was in accordance with Morsy et al. (2014) (9), who stated that GM was able to induce activation of the iNOS enzyme and increase NO output in renal tissue.

In addition, Abd-Elhamid et al. (2018) (30) reported that there has been a negative association in experimental models between eNOS immunoexpression and the degree of renal pathology. In this study, histopathological examination showed that renal structural pathological abnormalities occurred in gentamicin-administered rats with glomerulus and

tubules. Compared to normal rats, the gentamicin group demonstrated diffuse acute renal tubules injury with intratubular hyaline casts. Increased interstitium tissue indicated marked tubular damage associated with chronic inflammatory cells infiltration. Glomerular atrophy noted that the surrounding diffuse acute renal tubules necrosis associated with hydropic degeneration in few tubules. The interstitial tissue showed lymphoplasmacytic infiltrates, with marked dilated congested cortical capillaries. These histopathological changes in tubules and glomeruli are similar to changes as described by Sardana et al. (2015) (33) as rats received nephrotoxic dose of gentamicin 100 mg/kg/day, i.p. once daily for 14 days.

Tadalfil has renoprotective effect as reported in previous studies as (18). Our results showed that tadalafil significantly decreased serum urea, serum creatinine, albumin/creatinine ratio, with significant increase in creatinine clearance and urinary creatinine in gentamicin treated rats. This is in accordance with previous studies, Ozbek et al. (2015) (34) showed that acute tadalafil administration caused a significant decrease in serum creatinine and blood urea nitrogen (BUN) levels, thus protecting the rat kidney from contrast-induced nephropathy.

Our results demonestrated that tadalafil reduced albuminuria in gentamicin treated rats. This is in accordance with Elhawary and Abdullah. (2017) (36) who showed that tadalafil decreased albuminuria in diabetic nephropathy and enhanced renal status.

By improving c-GMP levels, selective PDE-5 inhibitors can also inhibit the activity and expression of NADPH oxidase, which is considered a source of ROS production (37). Previously, tadalafil pretreatment in rats led to a significant decrease in MDA by reducing the pathway of lipid peroxidation. Tadalafil also significantly increased the activity of SOD enzymes in the ischemia/reperfusion group of rats (38).

These previous findings are consistent with the current research in which tadalafil induced significant increases in CAT and SOD renal tissue activities, as well as concomitant decreases in MAD renal tissue content

in rats treated with gentamicin. As stated in previous studies, this strongly suggests the potential antioxidant and anti-lipoperoxidative effects of tadalafil.

In the present study tadalafil reduced increased iNOS expression and caused increase in eNOS expression in gentamicin-treated rats. It also decreased nitric oxide production in gentamicin- treated rats.

This in accordance with previous results. Tadalafil (10 mg/kg/day; p.o) has been shown to partially protect against arthritis caused by the complete adjuvant of Freund (CFA) by oxidant/antioxidant balance improvement. Tadalafil significantly decreased markers of oxidative stress as iNOS, NO, while improved protein expression of eNOS. It was previously studied that cGMP could decrease the level of tumor necrosis factor-alpha and, subsequently, NO in inflamed joints released by neutrophils via iNOS. The regulation of the production of NO may therefore be due to the function of cGMP that accumulates when a PDE-5 inhibitor is administered (39).

Tadalafil is phophodieterase 5 inhibitor that improves circulating inflammatory cytokines, reverses oxidant/antioxidant dysfunction and ultimately has an overall protective impact on renal tissue from kidney damage associated with Escherichia coli-induced pyelonephritis. Tadalafil administration significantly decreased the levels of nitric oxide. Consequently, a novel therapeutic target for pyelonephritis may be a phophodieterase 5 inhibitor (40).

It was concluded that tadalafil has reno-protective effects in AKI after nephron-sparing surgery following renal artery clamping. Tadalafil resulted in a significant reduction in urine levels of KIM-1 in the tadalafil-treated group relative to control group (35). This is in line with the present study where tadalafil caused signficant decrease in urine KIM-1 level in gentamicin treated rats.

In addition, the prevalent normal renal morphology with only occasional tubular degenerative changes and decreased interstitial inflammatory infiltrates demonstrated this nephroprotective effect of tadalafil.

These findings are consistent with previous results stating that the protective effect of tadalafil was corroborated by unremarkable

histological alterations in the tubular-interstitial architecture of 2 mg/kg and 5mg/kg of tadalafil pretreated rats prior to treatment with cisplatin (18).

5. Conclusions, Recommendations:

In conclusion, treatment with tadalafil attenuates gentamicin nephrotoxicity in rats partially by improving oxidative stress by preserving catalase and superoxide dismutase activity and inhibiting malondialdehyde in the renal cortex, as well as by inhibiting iNOS expression and inducing eNOS development.

In order to be able to fully investigate the protective effect of tadalafil on gentamicin nephrotoxicity, further studies with different doses and for a longer period of tadalafil and a larger sample size are required. More studies aimed at the side effects of tadalafil should also be done.

6. References:

1. Nagib RM. Inhibitory Effects of Grape Seeds Powder, Extract and Oil on Gentamicin Induced Nephrotoxicity in Rats (2014). Egypt Journal of Nutrition and Health;9(1):1.
2. Wargo K and Edwards J. Aminoglycoside-Induced Nephro-toxicity (2014). Journal of Pharmcological Practice;27(6):573.
3. Adil M, Kandhare AD, Dalvi G, Ghosh P, Venkata S, Raygude KS, Bodhankar SL. Ameliorative effect of berberine against gentamicin induced nephrotoxicity in rats via attenuation of oxidative stress, inflammation, apoptosis, and mitochondrial dysfunction (2016). Journal of Renal failure;38(6):996.
4. Hafez LO, Ali FA, El-Ghoneimy AA and Abdel-Aziz MI. Nephro-Protective effect of wheat germ oil on gentamicin-induced acute nephrotoxicity in wistar albino rat (2019). SVU-International Journal of Veterinary Sciences;2(1):51.
5. Cairoli C, Reyes LA, Henneges C and Sorsaburu S. PDE5 inhibitor treatment persistence and adherence in Brazilian men: post-hoc analyses from a 6-month, prospective, observational study (2014). International Brazilian Journal of Urology;40(3):390.
6. Tomita N, Hotta Y, Naiki-Ito A, Hirano K, Kataoka T, Maeda Y, Takahashi S, Kimura K. The phosphodiesterase 5 inhibitor tadalafil has renoprotective effects in a rat model of chronic kidney disease (2020). Journal of Reproductive Physiology;8(17):14556.

7. Okamoto K, Kurita M, Yamaguchi H, Numakura Y, Oka M. Effect of tadalafil on chronic pelvic pain and prostatic inflammation in a rat model of experimental autoimmune prostatitis (2018). Journal of Prostate;78(10):707.
8. Brock G, Ni X, Oelke M, Mulhall J, Rosenberg M, Seftel A, D'Souza D and Barry J. Efficacy of continuous dosing of tadalafil once daily vs. tadalafil on demand in clinical subgroups of men with erectile dysfunction: A descriptive comparison using the Integrated Tadalafil Databases (2016). The Journal of Sexual Medicine;13(5):860.
9. Morsy MA, Ibrahim SA, Entesar FA, Kamel MA, Rifaai RA and Hassan MK. Sildenafil Ameliorates Gentamicin-Induced Nephrotoxicity in Rats: Role of iNOS and eNOS (2014). Journal of Toxicology;2014.
10. Adeneye A and Benebo AS. Chemopreventive Effect of Tadalafil in Cisplatin-Induced Nephrotoxicity in Rats (2016). Nigerian Journal of Physiological Sciences;31(1):1.
11. AL-kuraishy HM, AL-Gareeb AI and Rasheed HA. Antioxidant and anti-inflammatory effects of Curcumin contribute into attenuation of acute Gentamicin -induced nephrotoxcity in rats (2019). Asian Journal of Pharmaceutical and Clinical Research;2(3):30875.
12. Kampmann JP and Hansen JM. Glomerular filtration rate and creatinine clearance (1981). British Journal of Clinical Pharmacology;12(1):7.
13. Hosaka EM, Santos OFP, Seguro AC and Vattimo MFF. Effect of cyclooxygenase inhibitors on gentamicin-induced nephrotoxicity in rats (2004). Brazilian Journal of Medical and Biological Research;37(7):979.
14. Sahu BD, Tatireddy S, Koneru M, Borkar RM, Kumar JM, Kuncha M, Srinivas R and Sistla R. Naringin ameliorates gentamicin-induced nephrotoxicity and associated mitochondrial dysfunction, apoptosis, and inflammation in rats: possible mechanism of nephroprotection (2014). Journal of Toxicology and Applied Pharmacology;277(1):8.
15. El-Kashef DH, El-Kenawi A, Suddek GM and Salem HA. Protective effect of allicin against gentamicin-induced nephrotoxicity in rat (2015). Journal of Internternational Immunopharmacology;29(2):679.
16. Salama SA, Arab HH and Maghrabi IA. Troxerutin down-regulates KIM-1,

modulates p38 MAPK signaling, and enhances renal regenerative capacity in a rat model of gentamicin-induced acute kidney injury (2018). Journal of Food &Function;9(12):6632.

17. Ghaznavi H, Mehrzadi S, Dormanesh B, Tabatabaei SM, Vahedi H, Hosseinzadeh A, Pazoki-Toroudi H, Rashidian A. Comparison of the Protective Effects of Melatonin and Silymarin Against Gentamicin-Induced Nephrotoxicity in Rats (2016). Journal of Evidence-Based Integrative Medicine;21(4):49.
18. Adeneye A and Benebo AS. Chemopreventive Effect of Tadalafil in Cisplatin-Induced Nephrotoxicity in Rats (2016). Nigerian Journal of Physiological Sciences;31(1):1.
19. Azouz AA, Saleh E and Abo-Saif AA. Aliskiren, tadalafil, and cinnamaldehyde alleviate joint destruction biomarkers; MMP-3 and RANKL; in complete Freund's adjuvant arthritis model: ownregulation of IL-6/JAK2/STAT3 signaling pathway (2020). Saudi Pharmaceutical Journal: SPJ: the official publication of the Saudi Pharmaceutical Society;28(9):1101.
20. Hejazi S, Kasebzadeh M and Tagdisi A. The Effect of Gentamicin Nephrotoxicity in Newborn Mice at Breastfeeding (2018). International Journal of Morphology;1(36):2.
21. Jaikumkao K, Pongchaidecha A, Chattipakorn N, Chatsudthipong V, Promsan S, Arjinajarn P and Lungkaphin A. Atorvastatin improves renal organic anion transporter 3 and renal function in gentamicin-induced nephrotoxicity in rats (2016). Experimental Physiology;101(6):743.
22. Balakumar P, WitnessKoe WE, Gan YS, JemayPuah SM, Kuganesswari S, Prajapati SK, Varatharajan R, Jayachristy SA, Sundram K and Bahari MB. Effects of pre- and post-treatments with dipyridamole in gentamicin-induced acute nephrotoxicity in the rat (2017). Journal of Regulatory Toxicology and Pharmacology;84:35.
23. Ehsani V, Amirteimoury M, Taghipour Z, Shamsizadeh A, Bazmandegan G, Rahnama A, Khajehasani F and Fatemi I. Protective effect of hydroalcoholic extract of Pistacia vera against gentamicin-induced nephrotoxicity in rats (2017). Journal of Renal Failure;39(1):519.
24. Randjelovic P, Veljkovic S, Stojiljkovic N, Sokolovic D and Ilic I. Gentamicin nephrotoxicity in animals: Current knowledge and future perspectives

(2017). Journal of Experimental and Clinical Sciences;16:388.

25. Diamond JR and Yoburn DC. Nonoliguric Acute Renal Failure (1982). Journal of Archives of Internernal Medicine;142(10):1882.
26. Choi HM, Kim SC, Kim MG, Jo SK, Cho WY, and Kim HK. Etiology and outcomes of anuria in acute kidney injury: a single center study (2015). Journal of Kidney Research and Clinical Practice;34(1):13.
27. Dungca NT. Protective effect of the methanolic leaf extract of Eclipta alba (L.) Hassk. (Asteraceae) against gentamicin-induced nephrotoxicity in Sprague Dawley rats (2016). Journal of Ethnopharmacology;184:18.
28. Chen Q, Cui Y, Ding G, Jia Z, Zhang Y, Zhang A and Huang S. PEA3 protects against gentamicin nephrotoxicity: role of mitochondrial dysfunction (2017). American Journal of Translational Research;9(5):2153.
29. Abdelrahman RS. Protective effect of apocynin against gentamicin-induced nephrotoxicity in rats (2018). Human & Experimental Toxicology Journal;37(1):27.
30. Abd-Elhamid TH, Elgamal DA, Ali SS, Ali FE, Hassanein EH, El-Shoura EA and Hemeida RA. Reno-protective effects of ursodeoxycholic acid against gentamicin-induced nephrotoxicity through modulation of NF-κB, eNOS and caspase-3 expressions (2018). Journal of Cell Tissue Research;374(2):367.
31. Mehan S, Dudi R and Kalra S. Renoprotective effect of corosolic acid in gentamicin-induced nephrotoxicity and renal dysfunction in experimental rats (2017). Journal of Annals of Pharmacology and Pharmaceutics;2(12):1065.
32. Pavlakou P, Liakopoulos V, Eleftheriadis T, Mitsis M and Dounousi E. Oxidative stress and acute kidney injury in critical illness: pathophysiologic mechanisms biomarkers inter ventions, and future perspectives (2017). Journal of Oxidative Medicine and Cellular Longevity;2017.
33. Sardana A, Kalra S, Khanna D, Balakumar P. Nephroprotective effect of catechin on gentamicin-induced experimental nephrotoxicity (2015). Journal of Clinical and Experimental Nephrology;19(2):178.
34. Özbek K, Ceyhan K, Koç F, Söğüt E, Altunkaş F, Karayakalı M, Çelik A, Kadı

H, Köseoğlu RD, Önalan O. The protective effect of single dose tadalafil in contrast-induced nephropathy: an experimental study (2015). Anatolian Journal of Cardiology;15(4):306.

35. Georgiadis G, Zisis IE, Docea AO, Tsarouhas K, Karavitakis M, Fragkiadoulaki I, Mavridis C, Calina D, Stratakis S, Stylianou K, Tsitsimpikou K, Sofikitis N, Tsatsakis A and Mamoulakis C. Current Concepts on the Reno-Protective Effects of Phosphodiesterase 5 Inhibitors in Acute Kidney Injury: Systematic Search and Review (2020). Journal of Clinical Medicine;9(5):1284.
36. Elhawary AM and Abdullah OM. Protective effect of tadalafil in diabetic nephropathy induced rat model (2017). International Journal of Basic and Clinical pharmacology;6(4):711.
37. Erol B, Turker T, Tok A, Bektas S, Mungan G, Ozkanli S, Karakas B, Tokgoz H, Akduman B and Mungan A. The protective effects of tadalafil on renal damage following ischemia reperfusion injury in rats (2015). The Kaohsiung Journal of Medical Sciences;31(9):454.
38. Altaş M, Aras M, Meydan S, Nacar E, Ulutaş KT, Serarslan Y and Yılmaz N. Effects of tadalafil on ischemia/reperfusion injury in rat brain (2014). Journal of Acta Neurologica Belgica;114(1):33.
39. Azouz AA, Saleh E and Abo-Saif AA. Aliskiren, tadalafil, and cinnamaldehyde alleviate joint destruction biomarkers; MMP-3 and RANKL; in complete Freund's adjuvant arthritis model: ownregulation of IL-6/JAK2/STAT3 signaling pathway (2020). Saudi Pharmaceutical Journal: SPJ: the official publication of the Saudi Pharmaceutical Society;28(9):1101.
40. Zhu CY, Liu M, Liu YZ, Li W, Zhai W, Che JP, Yan Y, Wang GC, and Zheng JH. Preventive effect of phosphodiesterase 5 inhibitor tadalafil on experimental post-pyelonephritic renal injury in rats (2014). Journal of Surgical Research;186(1):253.