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RESEARCH ARTICLE

Liraglutide Attenuates Gentamicin-Induced Nephrotoxicity in Rats by Reducing Oxidative Stress

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Abstract

Objective: Nephrotoxicity is a major complication of gentamicin (GEN), which is widely used in the treatment of severe Gram-negative infections. As we know, treatment with liraglutide has been shown to reduce oxidative stress. Therefore, we evaluated the potential protective effect of liraglutide against GEN-induced nephrotoxicity in rats.

Material And Methods: Twenty-eight rats were randomly divided into four groups: control group (Group 1); rats intraperitoneally injected with GEN (100 mg/kg/day; Group 2); rats treated with GEN plus distilled water (Group 3); and rats treated with GEN plus liraglutide (0.6 mg/kg/day; Group 4). After 15 days, the rats were sacrificed, their kidneys taken, and blood analysis performed. Tubular necrosis, interstitial fibrosis, and inducible nitric oxide synthetase (iNOS) scores were determined histopathologically in a part of the kidneys; malondialdehyde (MDA), reduced glutathione (GSH), E-cadherin and transforming growth factor β1 (TGF-β1) levels were determined in another part of kidneys.

Results: The GSH levels in renal tissue of only GEN-treated rats were significantly lower than others, and the administration of liraglutide to rats significantly increased the level of GSH. The group that was given GEN plus liraglutide had significantly lower MDA, TGF - β1, and E - cadherin levels than that given GEN alone. The rats treated with GEN+liraglutide indicated less severe tubular necrosis and their glomeruli maintained a better morphology compared to the GEN group. iNOS expression was higher in the liraglutide administrated group than the group that applied only GEN.

Conclusion: Liraglutide exerts protective effects on GEN-induced kidney damage by reducing oxidative stress in rat model.

Keywords: gentamicin, liraglutide, kidney

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INTRODUCTION

The kidney is a major organ that plays stunning roles in the human body. The primary duty of the kidney is to sustain the total body fluid volume, its composition, and acid-base balance. Many exogenous pollutants and chemical factors, including drugs, can alter kidney function(1). Gentamicin (GEN), a type of aminoglycoside, is used in the treatment of bacterial infections, but this drug is not renal parenchyma friendly. Nephrotoxicity, estimated to occur in approximately 10-20% of aminoglycoside treatments, is the most important complication of this drug (2) 40 mg/kg b.wt. Compared to the mitochondrial respiratory rates of 7 sham-control rats, State 3 (ADP dependent. Renal free radical production and accumulation causes glomerular congestion and the glomerular filtration rate decreases. This oxidative stress environment due to the rise of free radicals causes acute tubular necrosis(3).

Reactive oxygen species (ROS) are known to be significant mediators of GEN nephrotoxicity(4). In vitro and in vivo studies have shown that substances that reduce reactive oxygen metabolites are protective in GEN-induced renal failure(5). Lipid peroxidation (LPO) interfered with by ROS is a factor of cell destruction in various pathological conditions and indicates cellular damage which is widely used in the treatment of severe Gram-negative infections (6). Reactive oxygen species are important mediators of GEN-induced nephrotoxicity. Because of the strong antioxidant properties of pomegranate extract (PE). Moreover, the activation of proapoptotic and proinflammatory mediators would be stimulated by the superelevation of ROS, thus contributing to kidney damage induced by gentamicin (7).

Various pharmacological substances have been determined to have a potency in avoiding GEN-induced nephrotoxicity(8). Nevertheless, there is currently no clinically encouraging intervention to completely prevent or minimize the effect of GEN-induced nephrotoxicity. The medical world has been dealing with kidney protective antioxidant and anti-inflammatory treatments for a long time. The glucagon-like peptide-1 (GLP-1) analog liraglutide has been reported to reduce high glucose-induced oxidative stress, tumor necrosis factor (TNF) –α, and inflammatory reaction in human endothelial cells(9). Inhibition of protein kinase C (PKC) translocation, nuclear factor (NF) -B activation and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activation contribute to the anti-inflammatory and anti-oxidative effects of liraglutide(10). Plasma reactive oxygen compounds are an indicator of oxidative stress. Studies have shown that liraglutide provides significant reductions in plasma reactive oxygen compounds in patients with type 2 diabetes (11). There is also an anti-inflammatory effect of liraglutide. This effect has been demonstrated by experimental animal studies in the brains of rats with Alzheimer's disease and the hearts of diabetic rats (12).

Owing to these influences of liraglutide, in our study, the role of liraglutide in renal damage with GEN was investigated. In the present study, we aimed to find out whether liraglutide has any protective effect against GEN toxicity in an experimental animal study. Since there is no study investigating the protective effect of liraglutide against GEN-induced nephrotoxicity in the literature, our study is the first study on this subject.

MATERIAL AND METHODS

Rats

The experimental study was done on all 28 male Wistar-Albino rats, weighing between 200-300 gr. The rats were housed in clean plastic cages at 20-22°C constant temperature and in a humidity-controlled facility with a stable 12-hour light/dark cycle. During the experiment, the rats were given enough water and rat feed with free access. Ethics committee approval was obtained by applying to the Istanbul University Animal Experiments Local Ethics Committee before the experiment (Approval number: 06/06/2018-147577).

Medicaments

GEN (Gentreks, Bilim Pharmaceutical, Istanbul, Türkiye), and Liraglutide (Victoza®, Novo Nordisk, Plainsboro, New Jersey, USA) were purchased from a random pharmacy. GEN was injected intraperitoneally at a dose of 100mg/kg/day. Liraglutide was administered at a dose of 0.3 mg/kg every 12 hours subcutaneous injection.

Experimental Design

After a quarantine period of seven-day-long, 28 animals were indiscriminately divided into 4 even groups, every consisting of seven rats as follows: (1) control group; (2) GEN injected group for 14 consecutive days intraperitoneally (100 mg/kg/day); (3) GEN plus %0.9 saline-treated group for 14 consecutive days subcutaneously and (4) GEN plus liraglutide treated group (0.6 mg/kg/day liraglutide was administered immediately after injection of GEN) for 14 consecutive days. All rats were processed for 14 consecutive days. After 15 days, the rats were sacrificed by taking blood

from the heart under anesthesia, blood analysis performed, and their kidneys taken. Histopathologically, interstitial fibrosis scores and tubular necrosis were investigated in a part of renal tissues; E-cadherin, reduced glutathione (GSH), malondialdehyde (MDA), and transforming growth factor β1 (TGF-β1) levels were measured in another part of renal tissues. Sodium (Na+), potassium (K+), creatinine, and urea levels were measured as a part of blood analysis.

Biochemical Assays

On day 15, the rats were anesthetized by using xylazine/ketamine (10/50 mg/kg, i.p.) and killed. 24-hour urine collections were acquired in normal cages one day before the rats were killed. Kidney tissues were split into 2 pieces for microscopic inspection and biochemical analysis. Blood was taken from the hearts of rats and Na +, K +, urea, and creatinine levels were measured. MDA, reduced GSH, E-cadherin, and Transforming growth factor beta-1 (TGF-β1) were measured in the renal cortical tissues. For the determination of MDA and GSH in rat kidney tissue, the tissues were weighed, 1/20 (weight/volume) phosphate buffer saline was added (0.1 M / pH 7.4), and homogenized with a tissue homogenizer. Homogenates were centrifuged at 3500rpm for 15 minutes, the supernatants were separated and stored at -80 ° C until the time to be analyzed. MDA levels were assayed spectrophotometrically. MDA is referred to as thiobarbituric acid reagent. MDA was measured with thiobarbituric acid at 532 nm. In the measurement of GSH, the method based on the use of Ellmann's reagent was used and spectrophotometrically measured. Serum creatinine and urea levels were measured by using Beckman Coulter diagnostic kits. Na+ and K+ values were measured by ion-selective electrode method using Beckman Coulter AU5821 autoanalyzer.

Histopathological Examinations

Veterinary pathologist performed the evaluations . Kidney tissue samples obtained from rats were routinely processed, embedded in paraffin blocks, and cut in a manual rotary microtome (Leica RM2255). Sections were investigated under a light microscope (Olympus BX50) after being stained with Hematoxylin & Eosin (H&E). Histopathological evaluation was made according to the degree of tubular damage and the intensity of inflammatory cell infiltration. If tubular damage is less than 25%, mild (1); If the affected tubule ratio is 25-50%, it is medium (2); If more than 50%, it is scored as severe (3). To evaluate inflammatory cell infiltration, 5 random areas were selected at 40x magnification for each kidney section, and scoring for neutrophils and mononuclear leukocytes were done separately. We used Masson's trichrome to assess the degree of interstitial fibrosis. The interstitial area was stained green with Masson's trichrome. Then, an image analyzer was then used (Leica; Leica Micros Imaging Solutions, Cambridge, UK), and 5 pieces were randomly selected from any kidney sample and analyzed. After that, the Banff classification was used to score the degree of interstitial fibrosis and mononuclear cell infiltration (13).

Immunohistochemical Staining

All immunohistochemical evaluations were done double-blinded. Kidney sections taken on positively charged slides for immunohistochemical (IHC) labeling with inducible nitric oxide synthetase (iNOS) primary antibody were stained following the manufacturer's instructions from which the antibody was supplied. Deparaffinized sections were passed through 100, 96, 80, and 70% alcohol series and distilled water, respectively. Heat treatment was applied with citrate buffer solution (pH 6.0) in a microwave oven for 20 minutes at 750W. Then, it was incubated for 10 minutes with a 0.3% hydrogen peroxide solution in methanol to block the endogenous peroxidase activity. Anti-iNOS (PA516855, 1/100 dilution, Invitrogen) antibody was then applied to the sections that were kept at room temperature for 10 minutes by dropping the protein block solution in a humid cabinet and incubating for 90 minutes at room temperature. After the primary antibody incubation, the reaction was completed with a secondary antibody kit (Expose Mouse and Rabbit Specific HRP / DAB Detection IHC Kit, Cat no. Ab80436, Abcam) based on the micro polymer principle. The sections were incubated at room temperature for 10 minutes with Horseradish Peroxidase (HRP) conjugated secondary antibody. It was labeled with diaminobenzidine (DAB). Floor painting with Mayer hematoxylin has been completed. Sections were washed with Tween 20-added phosphate buffer solution (pH 7.4) after all incubation steps until background staining, except between protein blocking and primary antibody steps. IHC staining was examined with a light microscope and the immune reaction was scored according to the ratio of marked tubular epithelial cells and the intensity of staining. If the rate of marked tubular epithelial cells is 0% (0); Light up to 30% (1); 30-60% medium (2); If more than 60%, it was determined as severe (3). Positive reaction intensity was rated as, no reaction (0); light (1); medium (2); severe (3). The final score is determined by multiplying these two scores. Results are expressed as negative (0); mild (1-3); medium (4-6); and strongly positive

(7-9)(14).

Statistical Analysis

The p-value of < 0.05 was accepted as statistically significant. Continuous variables of entire groups were indicated as mean values ± standard deviation (SD). The distribution was examined with the Shapiro-Wilk test. Histopathological data were evaluated using the chi-square test. Biochemical data were analyzed using the Kruskal-Wallis test. In the case of statistical significance between groups, the Mann-Whitney U test was used to determine whether it was significant for the two groups. Bonferroni adjustment for multiple comparisons was applied. P<0.0083 was considered to indicate a significant difference, since the number of groups was four, and the significance level was 5%.

RESULTS

There were no signs of death or visible toxicity in the rats. The biochemical, histopathological, and immunohistochemical outcomes were analogous for the control and liraglutide-treated groups, therefore, we determined to evaluate them indiscriminately and to declare only the control group.

Urinary Volume

It was observed that the highest amount of urine was collected from the GEN-treated rats. This was statistically significant (p < 0.01). This indicated the presence of GEN-induced polyuria. There was no difference in the liraglutide injected group compared to the control group, pointing out the preventive effect of liraglutide towards ATN (Table 1).

Biochemical Variables

The urea and creatinine values were found to be statistically significantly higher in the group of rats treated with GEN alone (p<0.05). With the addition of Liraglutide to the treatment of rats under GEN, a decrease in serum urea and creatinine levels was observed. Na+ and K+ values among the four groups had similarities (Table 1).

When MDA and GSH values were calculated, it was observed that the MDA value in the 4th group and the GSH value in the 3rd group were significantly lower than the other groups (p<0.05). Application of liraglutide after GEN treatment provided a statistically significant increase in GSH values (p < 0.05). When E-cadherin and TGF - β1 values were evaluated, it was observed that they were increased in the group treated with GEN. These values were statistically significant when compared with the control group. TGF - β1 and E - cadherin values decreased significantly after liraglutide injection (p<0.05). The details are shown in Table 2.

Histopathological and Immunohistochemical Results

While mild tubular damage such as granular and vacuolar degeneration was observed in the tubular epithelium in animals belonging to the control group (Figure 1), neutrophil or mononuclear cell infiltration was not detected in the intertubular, interstitial, and perivascular areas. Moderate and severe tubular necrosis, degeneration, dilatation, vacuolization in the tubular epithelium, epithelial hyperplasia, and mild interstitial and perivascular mononuclear cell infiltration were observed in the 2nd and 3rd groups. In the comparison between groups in Table-3, it was seen that tubular necrosis and interstitial fibrosis were more severe in the groups given GEN. In the group given GEN+Liraglutide, a similar histopathological appearance was observed in the control group. Leukocyte infiltration and iNOS were observed to be more severe in the groups that intervened with GEN, and when liraglutide was added to the treatment, similar results were obtained in the control group. (Figure 2 and Figure 3) (Table 3 and Table 4). Epithelial hyperplasia and mononuclear inflammatory cells were observed in the kidney tissues after liraglutide treatment. There was a decrease in the number of degenerative tubules and mononuclear inflammatory cells (Figure 4).

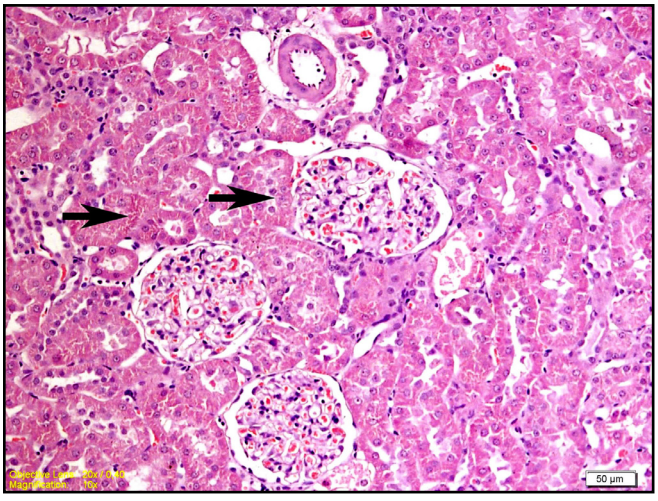

Figure 1. Degenerated, swollen, and granular-looking tubular epithelium (arrows), control group, Bar = 50 µm, H&E

Table 1. Effects of GEN alone and its combination with liraglutide on plasma urea, creatinine, Na^+, K^+, and 24-h urine volume levels in rats.

Parameters	Control (Group 1)	GEN (Group 2)	GEN+Ve (Group 3)	GEN+Liraglutide (Group 4)
Urea (mg/dl)	34±7,1	102±10,3[a]	101±5,1	42±10,2[b]
Creatinine (mg/dl)	0,42±0,1	1,89±0,9[a]	1,83±1,2	0,72±0,4[b]
Na^+ (mmol/L)	138±1,2	139±2,5	138,2±1,3	137±1,6
K^+ (mmol/L)	3,9±0,3	3,9±0,9	4±0,5	3,9±0,2
24-h urine volume (ml)	8,8±1,2	20,3±3,7[a]	21,1±3,2	8,9±1,9[b]

Notes: Values are expressed as mean±SD for seven rats in each group.

Groups: Control, GEN (gentamicin), GEN+Ve (gentamicin+vehicle), GEN+liraglutide(gentamicin+liraglutide)

[a]Significantly different from the control.

[b]Significantly different from the gentamicin group ($p<0.05$).

Table 2. Effects of liraglutide on rat kidney MDA, GSH, E-cadherin, and TGF-β1 levels.

Parameters	Control (Group 1)	GEN (Group 2)	GEN+Ve (Group 3)	GEN+Liraglutide (Group 4)
MDA (μM/mg)	16,5±5,8	26,4±4,3[a]	24,3±2,5	18±4,5[b]
GSH (μg/mg)	3,1±2	2±0,5[a]	1,81±0,9	2,9±0,7[b]
E-cadherin (ng/mg)	0,5±0,2	1,5±0,1[a]	1,5±0,3	0,6±0,3[b]
TGF-β1 (pg/mg)	2,5±0,7	8±1,7[a]	7,6±2,4	3,3±0,5[b]

GSH: reduced glutathione

MDA: malondialdehyde

TGF-β1: transforming growth factor β1

Notes: Values are expressed as mean±SD for seven rats in each group

[a]Significantly different from the control.

[b]Significantly different from the gentamicin group ($p<0.05$)

Table 3. Semiquantitative analysis of tubular necrosis, interstitial fibrosis in control, GEN, GEN + Ve, and GEN + liraglutide groups.

	Tubular necrosis						Interstitial fibrosis			
	n	none	+	++	+++		none	+	++	+++
Control	7	6	1	0	0		6	1	0	0
GEN	7	0	1	2	4		0	1	0	6
GEN + Ve	7	0	0	1	6		0	0	0	7
GEN + liraglutide	7	1	3	3	0		4	1	1	1

n: total number of rats each group , +: mild , ++: moderate , +++: severe tubular necrosis and interstitial fibrosis reported

Acute inflammation findings such as tubuler necrosis and interstitial fibrosis were highest in the ischemia-reperfusion group compared to the other groups.

Table 4. Comparison of leukocyte infiltration and iNOS between groups

	Leukocyte infiltration						iNOS			
	n	**none**	**+**	**++**	**+++**		**none**	**+**	**++**	**+++**
Control	7	6	1	0	0		2	2	2	1
GEN	7	0	1	2	4		1	3	2	1
GEN + Ve	7	0	0	2	5		1	2	3	1
GEN + liraglutide	7	2	3	2	0		1	1	4	1

n: total number of rats each group , +: mild , ++: moderate , +++: severe posivity fibrosis reported

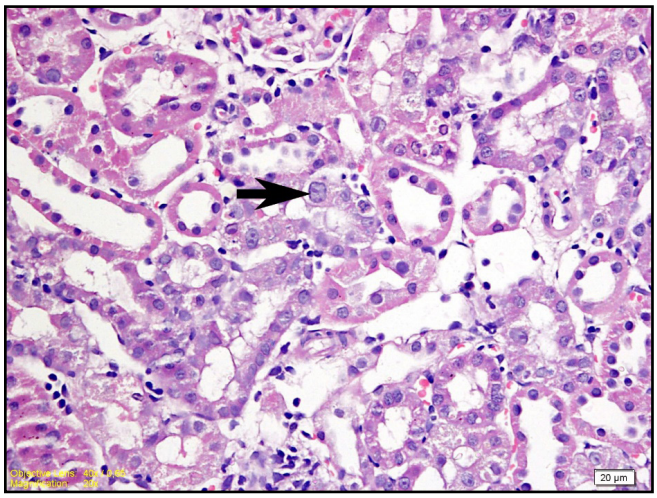

Figure 2. Degenerated, swollen, and granular-looking tubular epithelium, coarse nucleus structure (arrow), GEN group Bar = 20 µm, H&E

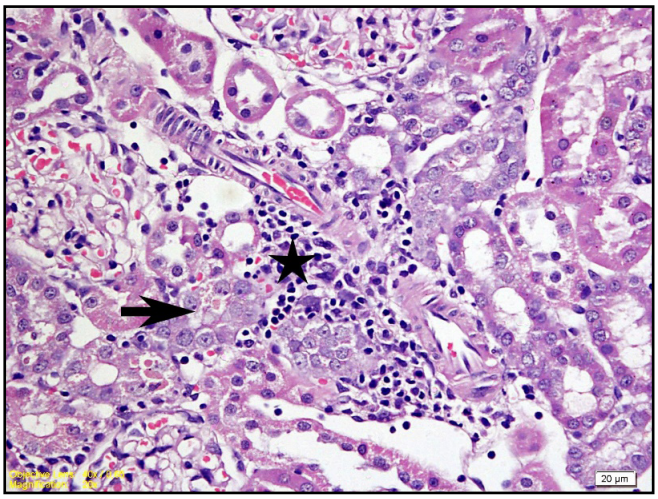

Figure 4. Epithelial hyperplasia areas (arrow), mononuclear inflammatory cells (stars), Gentamicin + Liraglutide group, Bar = 20 µm, H&E.

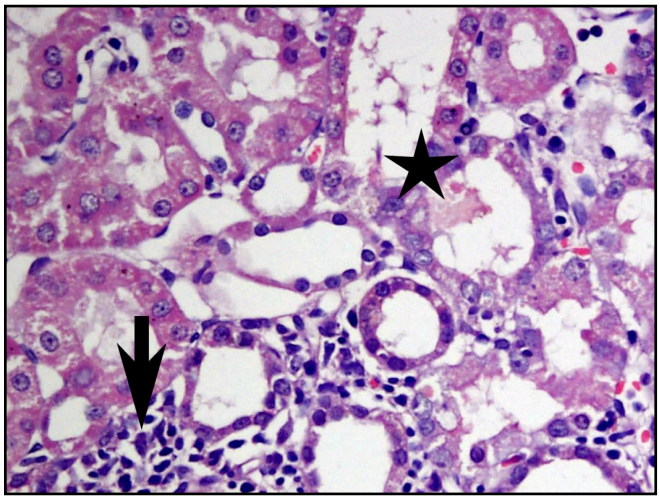

Figure 3. Degenerative tubules (stars), mononuclear inflammatory cells (arrow), GEN group, Bar = 20 µm, H&E

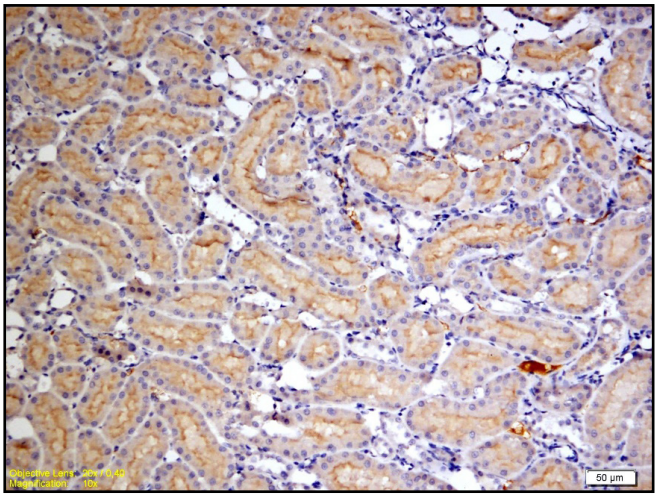

Figure 5. Slight anti-iNOS positive reaction, Control group, Bar = 50 µm, IHC

Varying degrees of positive reaction were found in all groups, including the control group in the immunohistochemical analysis (Figure 5). An anti-iNOS immune positive reaction was observed especially in proximal tubular epithelium. The reaction was rarely observed in the glomerular area. Especially in the gentamicin-applied group, since tubular damage was more severe than in the other groups (Figure 6), the immune reaction was found less due to the absence or necrosis of the epithelium. Tubular damage was moderate in group 4. iNOS expression was higher in group 4 than in the group that was applied only gentamicin (Table 4).

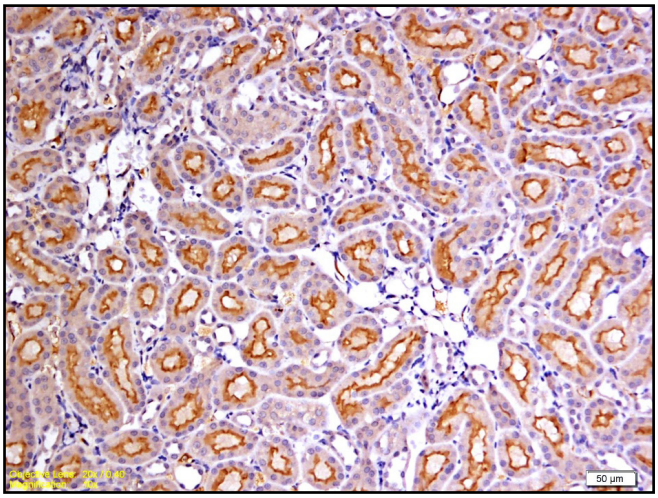

Figure 6. Strong positive anti-iNOS reaction showing intensity in proximal tubules, Gentamicin group, Bar = 50 µm, IHC

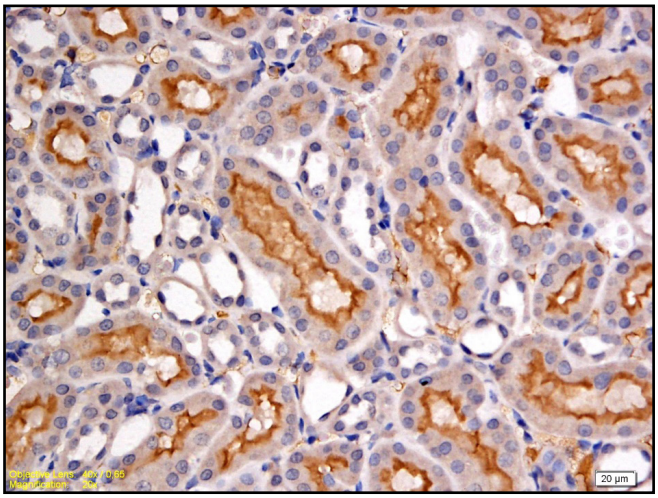

Figure 7. Widespread strong positive anti-iNOS reaction, liraglutide treated group, Bar = 20 µm, IHC

DISCUSSION

The kidneys are sensitive to damage from drugs. Aminoglycosides are one of the antibiotics with proven efficacy against gram-negative bacteria and are used clinically in daily practice, but nephrotoxicity may occur in its use. The tubular effect, triggered by drug accumulation in epithelial tubular cells, constitutes a major part of GEN-induced AKI. Ultimately, gentamicin causes acute tubular necrosis by causing glomerular obstruction, free radical production in the kidney, and decreased antioxidant protection mechanisms which may range from a mere loss of the brush border in epithelial cells to an overt tubular necrosis (15). Tubular cytotoxicity is the consequence of many interconnected actions, triggered by drug accumulation in epithelial tubular cells. Accumulation results from the presence of the endocytic receptor complex formed by megalin and cubulin, which transports proteins and organic cations inside the cells. Gentamicin then accesses and accumulates in the endosomal compartment, the Golgi and endoplasmic reticulum (ER). The use of certain antioxidant agents to reduce or prevent the effect of GEN nephrotoxicity is a reasonable consideration owing to the clear burden of ROS in GEN-induced kidney injury 40 mg/kg b.wt. Compared to the mitochondrial respiratory rates of 7 sham-control rats, State 3 (ADP) dependent (2).

Recently, it has been started to share data that liraglutide reduces oxidative stress caused by high glucose level and tumor necrosis factor (TNF) -α, in endothelial cells(9). Inhibition of PKC translocation, NADPH oxidase activation, and NF -B activation contribute to the anti-inflammatory and anti-oxidative effects of liraglutide(10). Besides, liraglutide reduces the levels of plasma reactive oxygen metabolites, one of the important indicators of oxidative stress (11).

The understanding of GEN nephrotoxicity is quite important clinically; such as in the presence of 1-2 liter urine output per day, an oliguric acute renal failure nephrotoxicity, which can be seen with azotemia(16). In the present study, the significant finding indicating the presence of polyuria due to GEN was that the 24-hour urine volume in the GEN group was the highest amount, while it was not different in the group treated with GEN + liraglutide. It showed the protective role of liraglutide against ATN. Measured creatinine and urea levels after treatment with GEN reflected kidney damage. Normalization of serum creatinine and urea levels after liraglutide administration showed that liraglutide restored renal function by protecting renal cells against the

GEN challenge. In this study, MDA levels, i.e. the indicators of lipid peroxidation of the membranes as a result of oxidative damage as well as both nuclear and mitochondrial DNA (17). Melatonin achieves this widespread protection by means of its ubiquitous actions as a direct free radical scavenger and an indirect antioxidant. Thus, melatonin directly scavenges a variety of free radicals and reactive species including the hydroxyl radical, hydrogen peroxide, singlet oxygen, nitric oxide, peroxynitrite anion, and peroxynitrous acid. Furthermore, melatonin stimulates a number of antioxidative enzymes including superoxide dismutase, glutathione peroxidase, glutathione reductase, and catalase. Additionally, melatonin experimentally enhances intracellular glutathione (another important antioxidant, were significantly increased after GEN administration. In parallel with this, GSH levels decreased in the renal tissue after GEN administration. GSH plays an important role in protecting the lipid and protein integrity in the cell membrane, as well as providing great protection from oxidative damage by participating in cellular defense systems (18). In the group treated with GEN+liraglutide, we found decreased MDA levels and increased GSH levels.

iNOS can be produced and detected by inducing secondary messengers in the inflammatory infection process(19). Particularly in the gentamicin-applied group, since tubular damage was more severe than the other groups, the immune reaction was found less due to the absence or necrosis of the epithelium in this study. iNOS expression was higher in the group treated with GEN + liraglutide than in the GEN group. Fibrosis and progressive tubular damage in the renal cortex have been demonstrated in previous studies. In our study, necrosis, degeneration, dilatation, and vacuolization of the tubular epithelium were evaluated histopathologically. All these were most severe in the GEN-treated group. This damage suggested that ROS formed as a result of oxidative stress caused by GEN. The kidney samples of the liraglutide-treated group had quite normal histological features except for slight desquamation and atrophy of the tubular epithelial cells. These findings strongly indicate that liraglutide may have a protective effect against the kidney from GEN-induced injury by improving the oxidant status.

Experimental animal studies are very important in establishing a new treatment modality as they provide the basis for clinical studies. To administer a drug to a person in a pathological situation, several stages are required. Experimental animal studies are one of these stages. In our study, a limited number of data were examined, so it would not be correct to obtain a definite result from a single study, however, when the data obtained from similar experimental studies are evaluated together, a basis for clinical studies can be formed.

This study had some limitations no evidence of long-term effects, the small sample, and the amount of biochemical and histopathological data examined is limited. Besides evaluated morphological semi-quantitative results, quantitative methods were not performed in the present study.

CONCLUSION

The results reported here indicate that liraglutide exerts antioxidant, anti-inflammatory and, antifibrotic effects on GEN-induced renal injury in rats by reducing oxidative stress. The observed protective effects can be attributed to the antioxidant properties of liraglutide. This study can be a good base for more studies aimed at finding the best therapy for this kind of pathology. Even if liraglutide could be protective against renal damage of GEN, it needs further research in larger animal groups.

Conflicts of interest: Authors have no conflicts of interest. The authors have nothing to disclose.

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Ethical Approval: Ethics committee approval was obtained by applying to the Istanbul University Animal Experiments Local Ethics Committee before the experiment (Approval number: 06/06/2018-147577). The study protocol conformed to the ethical guidelines of the Helsinki Declaration.

Author Contributions: Conception and design: Degirmentepe RB, Bozkurt M, Data acquisition: Otunctemur A, Yildirim F, Data analysis and interpretation: Erkoc M, Can O, Drafting the manuscript: Degirmentepe RB, Critical revision of the manuscript for scientific and factual content: Gurgen Ozturk H, Eraldemir FC, Hunc F Statistical analysis: Degirmentepe RB

REFERENCES

1. Otunctemur A, Ozbek E, Cekmen M, Cakir SS, Dursun M, Polat EC, et al. Protective effect of montelukast which is cysteinyl-leukotriene receptor antagonist on gentamicin-induced nephrotoxicity and oxidative

damage in rat kidney. Renal Failure. 2013;35(3):403–10. https://doi.org/10.3109/0886022X.2012.761040

2. Simmons CF, Bogusky RT, Humes HD. Inhibitory effects of gentamicin on renal mitochondrial oxidative phosphorylation. Journal of Pharmacology and Experimental Therapeutics. 1980;214(3):709–15.

3. Hur E, Garip A, Camyar A, Ilgun S, Ozisik M, Tuna S, et al. The effects of vitamin D on gentamicin-induced acute kidney injury in experimental rat model. International Journal of Endocrinology. 2013;2013. https://doi.org/10.1155/2013/313528

4. Banday AA, Farooq N, Priyamvada S, Yusufi ANK, Khan F. Time dependent effects of gentamicin on the enzymes of carbohydrate metabolism, brush border membrane and oxidative stress in rat kidney tissues. Life Sciences. 2008;82(9–10):450–9. https://doi.org/10.1016/j.lfs.2007.11.014

5. Pedraza-Chaverrí J, Maldonado PD, Medina-Campos ON, Olivares-Corichi IM, Granados-Silvestre MDLÁ, Hernández-Pando R, et al. Garlic ameliorates gentamicin nephrotoxicity: Relation to antioxidant enzymes. Free Radical Biology and Medicine. 2000;29(7):602–11. https://doi.org/10.1016/s0891-5849(00)00354-3

6. Cekmen M, Otunctemur A, Ozbek E, Cakir SS, Dursun M, Polat EC, et al. Pomegranate extract attenuates gentamicin-induced nephrotoxicity in rats by reducing oxidative stress. Renal Failure. 2013;35(2):268–74. https://doi.org/10.3109/0886022X.2012.743859

7. Dursun M, Sahin S, Besiroglu H, Otunctemur A, Ozbek E, Cakir SS, et al. Protective effect of nebivolol on gentamicin-induced nephrotoxicity in rats. Bratislava Medical Journal. 2018;119(11):718–25. https://doi.org/10.4149/BLL_2018_128

8. Balakumar P, Rohilla A, Thangathirupathi A. Gentamicin-induced nephrotoxicity: Do we have a promising therapeutic approach to blunt it? Vol. 62, Pharmacological Research. 2010. p. 179–86. https://doi.org/10.1016/j.phrs.2010.04.004

9. Shiraki A, Oyama J ichi, Komoda H, Asaka M, Komatsu A, Sakuma M, et al. The glucagon-like peptide 1 analog liraglutide reduces TNF-α-induced oxidative stress and inflammation in endothelial cells. Atherosclerosis. 2012;221(2):375–82. https://doi.org/10.1016/j.atherosclerosis.2011.12.039

10. Batchuluun B, Inoguchi T, Sonoda N, Sasaki S, Inoue T, Fujimura Y, et al. Metformin and liraglutide ameliorate high glucose-induced oxidative stress via inhibition of PKC-NAD(P)H oxidase pathway in human aortic endothelial cells. Atherosclerosis. 2014;232(1):156–64. https://doi.org/10.1016/j.atherosclerosis.2013.10.025

11. Okada K, Kotani K, Yagyu H, Ando A, Osuga J ichi, Ishibashi S. Effects of treatment with liraglutide on oxidative stress and cardiac natriuretic peptide levels in patients with type 2 diabetes mellitus. Vol. 47, Endocrine. 2014. p. 962–4.

12. Parthsarathy V, Hölscher C. The type 2 diabetes drug liraglutide reduces chronic inflammation induced by irradiation in the mouse brain. European Journal of Pharmacology. 2013;700(1–3):42–50. https://doi.org/10.1016/j.ejphar.2012.12.012

13. Kinugasa F, Noto T, Matsuoka H, Urano Y, Sudo Y, Takakura S, et al. Prevention of renal interstitial fibrosis via histone deacetylase inhibition in rats with unilateral ureteral obstruction. Transplant Immunology. 2010;23(1–2):18–23. https://doi.org/10.1016/j.trim.2010.02.003

14. Buyuklu M, Kandemir FM, Ozkaraca M, Set T, Bakirci EM, Topal E, et al. Benefical effects of lycopene against contrast medium-induced oxidative stress, inflammation, autophagy, and apoptosis in rat kidney. Human and Experimental Toxicology. 2015;34(5):487–96. https://doi.org/10.1177/0960327114542964

15. Quiros Y, Vicente-Vicente L, Morales AI, López-Novoa JM, López-Hernández FJ. An integrative overview on the mechanisms underlying the renal tubular cytotoxicity of gentamicin. Toxicological Sciences. 2011;119(2):245–56. https://doi.org/10.1093/toxsci/kfq267

16. Silan C, Uzun Ö, Çomunoğlu NÜ, Gokçen S, Bedirhan S, Cengiz M. Gentamicin-induced nephrotoxicity in rats ameliorated and healing effects of resveratrol. Biological and Pharmaceutical Bulletin. 2007;30(1):79–83. https://doi.org/10.1248/bpb.30.79

17. Reiter RJ, Acuña-Castroviejo D, Tan DX, Burkhardt S. Free radical-mediated molecular damage: Mechanisms for the protective actions of melatonin in the central nervous system. In: Annals of the New York Academy of Sciences. 2001. p. 200–15.

18. Ross D. Glutathione, free radicals and chemotherapeutic agents. Mechanisms of free-radical induced toxicity

and glutathione-dependent protection. Pharmacology and Therapeutics. 1988;37(2):231–49. https://doi.org/10.1016/0163-7258(88)90027-7

19. Chamberlain CG, Mansfield KJ, Cerra A. Nitric oxide, a survival factor for lens epithelial cells. Molecular Vision. 2008;14:983–91.