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Nephroprotective Effect of Alogliptin and L-Carnitine in Gentamicin -induced Toxicity in Male Albino Rats

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Abstract

The purpose of this investigation is the supportive functions of L-carnitine when co-administered with the DDP-4 inhibitor member and the nephroprotective impact of alogliptin in nephrotoxicity induced by gentamicin in male Wistar rats. A total number of 25 male Albino rats weighed 130.0±5.7 g, and divided into five equal groups, G1: (Control negative), G2: (Control positive, has nephrotoxicity), G3: (Treated group with L-carnitine), G4: (Treated with Alogliptin) and G5: (Treated with alogliptin and L-carnitine). The biochemical results of serum total proteins, albumin, urea, uric acid, and creatinine were significantly changed ($P \leq 0.05$) among different groups while serum globulin was not significant, and the mean values of these parameters showed significant variations in G5 than G3 and G4. The following measures were made in order to identify the oxidative/antioxidant cascades: glutathione peroxidase (GPx), catalase (CAT);, concentration of malondialdehyde (MDA) and Super Oxide Dismutase (SOD) and these parameters were showed significant changes ($P \leq 0.05$) among different groups and the mean value showed the significant of G5 than G3 and G4. The results of the histological and immunohistochemical analyses point to a possible function for alogliptin and L-carnitine in preventing renal tissue destruction in rat models of gentamicin-induced nephrotoxicity. According to the improvement of various biochemical indicators and oxidative state as well as the restoration of the renal structural integrity and function, treatment with alogliptin or L-carnitine protects the kidney through their antioxidant action. Better results than each medicine alone is seen when the two are combined.

KEYWORDS
Nephrotoxicity, L- carnitine, Alogliptin, Male Wistar Rats, Egypt

INTRODUCTION

Nephrotoxicity is defined as a rapid decline in kidney function brought on by the toxic effects of drugs and substances. There are numerous types, and some medications may have multiple negative effects on renal function. Nephrotoxic compounds are known as nephrotoxins. Nephrotoxicity should not be confused with the fact that some drugs need to have their dose adjusted for the diminished renal function because they are excreted mostly through the kidneys (Lucas *et al*., 2019).

Nephrotoxicity can occur due to a variety of processes, such as renal tubular toxicity, inflammation, glomerular destruction, crystal nephropathy, and thrombotic events. microangiopathy (Al-Kuraishy *et al*., 2019). The activity of endogenous incretin (glucagon-like peptide-1 and glucose-dependent insulinotropic polypeptide), which is a degrading enzyme of incretin, is enhanced by the use of dipeptidyl peptidase-4 (DPP-4) inhibitors, a novel class of oral hypoglycemic drugs. Through the increase of glucagon-like peptide-1, DPP-4 inhibitors also reduce glucagon production. Due to their minimal risk of hypoglycemia and other side effects, they are typically well tolerated (Karagiannis *et al*., 2012).

Alogliptin (ALO) benzoate, a recently created DPP-4 inhibitor, has received clinical approval in Japan for the treatment of type 2 diabetes (T2DM). An alogliptin was created using a structure-based approach and was found to have a high DPP-4 selectivity. Recent clinical trials have demonstrated that the efficacy and safety of alogliptin are comparable to those of other DPP-4 inhibitors. The clinical effectiveness and safety of alogliptin for the treatment of type 2 diabetes are the main topics of this revised review of a prior publication (Berhan and Berhan, 2013). L-carnitine (LC) is a naturally occurring substance that is created from the amino acids' lysine and methionine. It is produced primarily in the liver and kidney through endogenous biosynthesis, which accounts for 75% of its dietary sources. (Bremer, 1983; Aboubakr, 2020).

L-carnitine is required for the mitochondrial ß-oxidation of fatty acids to produce ATP (Furuno *et al*., 2001). As a result, LC can stop the damage that mitochondrial oxidative stress causes to the organelles and the apoptosis that it causes in certain cell types (Barhwal *et al*., 2007). Numerous organs, including the heart, colon, retina, and brain, were discussed in relation to the significant regulatory function of LC in the antioxidant processes. (Al-Majed *et al*., 2006; Cetinkaya *et al*., 2006; Sezen *et al*., 2008).

L-carnitine is a water-soluble antioxidant that is mostly taken from the human diet. It is an amino acid that is manufactured by the body from lysine and methionine (Gupta *et al*., 2018). Carnitine and its acyl derivatives have been found to have possible antioxidant properties (Sen *et al*., 2013). Additionally, carnitine

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demonstrated positive benefits on coronary heart disease, heart failure, and renal failure with increased oxidative stress (Calò *et al*., 2006; Sener *et al*., 2004).

A naturally occurring amino acid derivative known as 3-hydroxy-trimethyl-aminobutyric acid is known as levo-carnitine (L-carnitine), and it is frequently used as an antioxidant supplement. In the liver, kidneys, and brain, L-carnitine is generated endogenously (Karabulut *et al*., 2020). L-carnitine plays a crucial role in energy production by facilitating the entry of fatty acids into mitochondria, which increases mitochondrial activity (Tousson *et al*., 2019). The kidneys are where L-carnitine metabolism is primarily found. Most of the L-carnitine is reabsorbed back into the proximal tubules of the kidneys after renal excretion (Novakova *et al*., 2016).

This study was undertaken to evaluate the protective, antioxidant, and histological effects of L-carnitine and alogliptin on gentamicin (GNT) induced nephrotoxicity in albino rats.

MATERIALS AND METHODS

Experimental Animals

A total of 25 white albino male rats weighing 130±5.7 g were used in this study. Rats were obtained from the Faculty of Veterinary Medicine, Laboratory Animal Center, Zagazig University, Egypt. Prior to the experiment, they had two weeks of acclimatization period.

The Faculty of Veterinary Medicine at Zagazig University in Egypt's Ethics Review Committee gave its ethical blessing to the experimental design of the current study (Approval No. 20181120). All rats were fed a conventional, commercially balanced diet and were given unlimited access to water.

Chemicals

Gentamicin (Schering-Plough, Egypt, provided the drug Garamycin®). It was administered by intraperitoneal injection (I/P) and each vial contains 80 mg. A dose of 10 mg/kg body weight of alogliptin (Inhiglip®) was administered once daily per os and was purchased from Kima, Egypt. A dose of 50 mg/kg body weight of L-carnitine (Carnitol® 50 mg) was administered once daily per os and was purchased from Global in Egypt. The Biodiagnostics Company was used to obtain all biochemical analysis materials (Dokki, Giza, Egypt).

Experimental design

Male albino rats were randomly divided into 5 equal groups for the investigation (5 rats each). Saline was given orally to Group I once daily for 21 consecutive days as the control group. In groups II, III, IV, and V, gentamycin (80 mg/kg body weight) was administered intraperitoneally for 10 days straight (Zhang *et al*., 1999). While group II (control positive) was left untreated. For 21 consecutive days, Group III was given L-carnitine (50 mg/kg body weight) orally (Nimet *et al*., 2007), alogliptin (10 mg/kg body weight) was administered orally once daily to Group IV (Eric *et al*., 2011), and L-carnitine (50 mg/kg body weight) and alogliptin (10 mg/kg body weight) were given to Group V.

Sampling

After 24 h from the end of the experiment, retro-orbital plexus punctures were used to collect blood samples from each rat, which were then stored at room temperature in a slanted position for blood coagulation for 30 minutes before being centrifuged at 1200 g for 20 minutes to get serum. The serum was kept at -20°C until it was used for biochemical research.

The animals of all groups were decapitated humanely after blood was collected, and each rat had its kidneys removed before being rinsed with physiological saline. For histological and immunohistochemical analyses, kidney tissues were promptly preserved in 10% neutral buffered formalin for 48 hours. All rats (25), along with any remaining samples, were interred in a burial trench that had been carefully and hygienically prepared.

Serum biochemical studies

Total proteins in serum were measured according to Henry (1964), whereas serum albumin was assessed based on Walters and Gerarde (1970). The difference between total proteins and albumin was used to determine serum globulin. Additionally, the serum concentrations of urea, uric acid, and creatinine were measured using Bartels *et al*. (1972) and Paglia and Valentine (1967) to assess kidney performance.

Detection of oxidative/antioxidant cascades

The following measures were made in order to identify the oxidative/antioxidant cascades: glutathione peroxidase (GPx) (Aebi, 1984), catalase (CAT) (Esterbauer *et al*., 1982), and superoxide dismutase (SOD) (Suvarna *et al*., 2018) activities, and the concentration of malondialdehyde (MDA) (Suvarna *et al*., 2018) employing specialized diagnostic kits supplied from the Egyptian Biodiagnostics Company in various experimental groups.

Histological examination

Rat renal tissue samples fixed in formalin underwent processing in an automated tissue processor. Dehydration and a two-step initial fixation made up the procedure. Fixation involved immersing the tissue for 48 h in 10% buffered formalin, followed by 30 minutes of removing the fixative with distilled water.

The tissues were then dehydrated by passing them through a graded sequence of alcohol (70%, 90%, and 100%). The tissue was first subjected to 70% alcohol for 120 minutes, then to 90% alcohol for 90 minutes, and finally to two cycles of 100% alcohol, each lasting an hour.

The samples were then cleared in numerous changes of xylene after dehydration. It involved immersing tissue for an hour in a combination of 50% alcohol and 50% xylene, then immersing it in pure xylene for a further 1.5 hours.The samples were then imbedded and blocked out after being impregnated with molten paraffin wax. Hematoxylin and eosin (HE) was used to stain the paraffin slices (4-5 um) (Eissa *et al*., 1998). Staining was used to reveal any pathological alterations in the studied tissues, including circulatory disturbances, inflammation, degenerations, apoptosis, necrosis, and other pathological changes.

Immunohistochemical Studies

The conventional immunohistochemistry techniques were used (Cattoretti *et al*., 1992). The tissue pieces were routinely microwaved to distinguish the antigen's epitopes (Hsu *et al*., 1981).

Immunostaining involves two steps to demonstrate antigen in tissues. The primary antibody is first bound to the relevant antigen, and then a secondary or link antibody, collectively termed as the universal secondary antibody is used to see this reaction.

The secondary antibody induces an amplification of the reac-

tion with its linked enzyme, increasing the sensitivity of the test, whereas the main antibody affects the specificity of the response. To see the markers, the Biotin-Streptavidin (BSA) method was utilized (Kaloyanides, 1991). Since diaminobenzidine (DAB) enables a permanent preparation, it was utilized as a chromogen. It was counterstained with hematoxylin.

Statistical analysis

Utilizing SPSS, a statistical analysis was carried out (Version 26.0; SPSS Inc., Chicago, IL, USA). One-way ANOVA was used to analyse the significant differences between groups, and the Duncan test was used as a post hoc analysis. P <0.05 was regarded as significant. Results were presented as mean SEM.

RESULTS AND DISCUSSION

Serum Biochemical Analysis

Table 1 displays the results of blood biochemical assays following intraperitoneal administration of GNT as well as the protective effects of L-carnitine and/or alogliptin. Serum renal product indicators (urea, creatinine, and uric acid) showed a significant increase (P<0.05) in GNT-impaired rats compared to the untreated control group. Treatment with 50 and 10 mg/Kg B.W., of L-carnitine and/or alogliptin, respectively, reversed the alterations in the examined blood parameters.

Using a rat model of GNT-induced nephrotoxicity, this study looked at the potential renoprotective effects of alogliptin and/or L-carnitine. Antibacterial medicine has historically used aminoglycosides. An antibiotic known as an aminoglycoside, *Micromonospora purpurea* is the source of gentamicin. Most Gram negative bacterial infections that are fatal are resistant to it (Nale *et al*., 2012).

Gentamicin is a crucial medication used to treat many illnesses in chickens and other animals (e.g., colibacillosis and salmonellosis) (Giurov, 1986). Antibiotic-related adverse events are typically brought about by one of three mechanisms: an inflated response to the pharmacological effects of the medication, an immune system response to the medication or its metabolites, or toxic consequences of the medication or its metabolites. According to the current study, giving rats an intraperitoneal injection of GNT for 10 days straight at a dose of 80 mg/kg body weight significantly impaired their kidney function as seen by elevated plasma levels of urea and creatinine, which are supported by past studies (Reddy *et al*., 2012; Al-Qasoumi, 2013)

When compared to serum levels obtained from normal rats, the results likewise showed a significant decrease in albumin and total protein concentrations. The complex phenomena of nephrotoxicity brought on by GNT is distinguished by a rise in plasma levels of creatinine and urea as well as severe proximal renal tubular necrosis, which is then followed by worsening and renal failure. Reactive oxygen species are thought to be connected to the toxicity of aminoglycosides, especially GNT (Ihsan, *et al*., 2010).

Concurrent administration of LC or ALO stopped GNT-induced increases in serum creatinine, uric acid, and urea levels. Treatment with both drugs successfully increased the reduced blood total proteins and albumin levels in GNT-administered mice. The modification of renal histological distresses brought on by GNT and the augmentation of the antioxidant defense system are linked to this improvement in kidney function metrics.

The outcome of the LC+GNT treated group in this study is consistent with Tunez, *et al*., (2007) and Aboubakr *et al*. (2020). They showed that LC decreases oxidative stress, damage from thioacetamide and tilmicosin, liver and kidney enzyme activity, and oxidative stress in rats. These findings were supported by histopathology and immunohistochemistry data. In the same vein, serum total protein, albumin, and globulin concentrations may have reduced significantly (P<0.05), according to Elkomy *et al*. (2020). The oxidation of fatty acids in the mitochondria to make ATP requires LC, a natural vitamin (Tunez, *et al*., 2007). So, it has antioxidant properties and plays protective roles against oxidative stress in various tissues, including liver and kidney (Cayir *et al*., 2009). Due to its antioxidant impact and ability to effectively act as a free radical scavenger, LC may be able to dramatically enhance the biochemical parameters of the liver and kidneys, and protecting membrane permeability (Augustyniak and Skrzy-

Table 1. Evaluation of treatments on serum biochemical parameters in rats (n = 5) in different experimental groups.

Parameters Groups	T. protein (g/dl)	Albumin (g/dl)	Globulin (g/d)	Urea (mg/dl))	Creatinine (mg/dl)	Uric Acid (mg/dl)
Control (-ve)	5.75± 0.04^{a}	2.80± 0.31^{a}	2.36±0.04^{a}	57.47 ± 3.05^{c}	0.58 ± 0.01^{c}	3.32± 0.22^{c}
Control (+ve)	3.30 ± 0.12^{d}	1.07 ± 0.29^{c}	2.11 ± 0.17^{a}	72.91 ± 4.16^{a}	0.81 ± 0.02^{a}	5.76 ± 0.35^{a}
L-carnitine (LC)	4.01 ± 0.15^{c}	1.49 ± 0.07^{b}	2.33± 0.09^{a}	70.41 ± 2.39^{ab}	0.73± 0.01^{ab}	4.47 ± 0.06^{b}
Alogliptin (Alg)	4.62 ± 0.18^{c}	1.58 ± 0.48^{b}	2.65± 0.47^{a}	64.94 ± 1.35^{b}	0.71 ± 0.01^{ab}	4.80 ± 0.09^{b}
LC+ Alg	5.37 ± 0.06^{ab}	2.18 ± 0.45^{a}	2.79±0.50^{a}	61.22 ± 2.55^{c}	0.64 ± 0.06^{b}	3.54 ± 0.08^{c}

LC, L carnitine at dose of 50 mg/Kg; Alogliptin (Alg) at dose of 10 mg/Kg.
Data are expressed as the mean ± SE. Within each row, mean with different letters are significantly different at p < 0.05.

Table 2. Evaluation of oxidative stress markers in kidney tissues in rats (n = 5). in different experimental groups.

Parameters Groups	SOD (IU/ml)	Catalase (IU/ml)	GPX (IU/ml)	MDA (nmol/ml)
Control (-ve)	5.93 ± 0.17^{a}	69.00± 0.57^{a}	4.06± 33.9^{a}	1.95 ± 0.15^{d}
Control (+ve)	2.13 ± 0.18^{e}	36.00 ± 2.64^{d}	1.07 ± 19.9^{e}	7.93 ± 0.77^{a}
L-carnitine (LC)	3.52 ± 0.18^{d}	53.00 ± 0.57^{c}	2.02 ± 37.7^{d}	5.07 ± 0.08^{b}
Alogliptin (Alg)	4.06 ± 0.07^{c}	53.00 ± 1.52^{c}	2.33 ± 31.4^{c}	4.07 ± 0.11^{bc}
LC+ Alg	5.03 ± 0.03^{b}	59.33 ± 0.33^{b}	3.21 ± 38.2^{b}	3.16 ± 0.03^{c}

LC: L carnitine at dose of 50 mg/Kg; Alogliptin (Alg) at dose of 10 mg/Kg.
Malondialdehyde: MDA; Catalase: CAT; Glutathione Peroxidase: GPX; Superoxide dismutase: SOD.
Data are expressed as the mean ± SE. Within each row, mean with different letters are significantly different at p < 0.05.

dlewska, 2009). LC protects against mitochondrial toxicants and reduces oxidative stress (Barhwal *et al*., 2007).

Additionally, they promote ß-oxidation, which lessens the negative effects of free fatty acids (Furuno *et al*., 2001). One of the dipeptidyl peptidase-4 (DPP-4) inhibitors used to treat type 2 diabetes patients is alogliptin. The obtained data are consistent with Shima *et al*. (2019), who looked at the case of a 68-year-old man with type 2 diabetes, hypertension, and cerebral infarction. Treatment with ALO (25 mg/d) revealed that total serum protein and albumin levels gradually increased and improved, proteinuria and serum creatinine levels improved, and urinary excretion of 2-microglobulin and -D-glucosaminidase (NAG) was significantly decreased.

Assessment of oxidative stress in renal tissue

Renal catalase activity was significantly reduced ($P<0.05$) after receiving gentamicin at a dose of 80 mg/kg once day for 10 days. The activity of SOD was also decreased significantly. GNT administration in the same regimen caused a substantial drop in GPx activity ($P<0.05$). On the other hand, it led to a considerable rise in MDA activity (P 0.05). Renal CAT, SOD, and GPx levels significantly increased during treatment with LC and ALO, but MDA levels significantly decreased when compared to the group receiving GNT. As shown by a high significant drop in the antioxidant enzyme activities of CAT, SOD, and GPx and a high significant increase in MDA generation, the gentamicin-treated group caused oxidative stress in the rat kidneys. It was discovered that giving rats gentamicin increased the formation of H_2O_2 in the renal cortical mitochondria, which increased the generation of superoxide anions (Walker *et al*., 1999), H_2O_2 and superoxide anion may combine to generate the reactive and erratic hydroxyl radical. When Fe^{2+} is present, this radical is created. Therefore, Fe^{2+} seems to be crucial for the gentamicin nephrotoxicity-related generation of reactive oxygen radicals. Renal cells use different antioxidant enzymes, including GPx, as a defensive strategy when oxygen radicals start to build up. Increased lipid peroxidation is caused by decreased activity of one or more antioxidant systems as a result of gentamicin's direct toxicity or volume depletion as a result of gentamicin treatment. The causes of gentamicin nephrotoxicity are a decreased level of intracellular glutathione and an increase of H_2O_2 and hydroxyl radicals (Bushra and Effa, 2007).

Because proximal tubule necrosis is the predominant site of drug accumulation and because GPx is almost exclusively produced in proximal tubular cells, the decrease in GPx activity in kidney as demonstrated by our results may be related to this condition (Pedraza-Chaverri *et al*., 2004). Additional studies by Yaman and Balikci (2010); Kamel *et al*. (2015) and Khan *et al*. (2011) supported our findings by demonstrating that administration of GNT led to a highly significant increase ($P<0.05$) in hepatic and renal MDA levels as well as a highly significant decrease in hepatic and renal catalase, SOD, and reduced glutathione activities when compared to healthy control animals. In a similar vein, Al-Qasoumi Giurov (1986) discovered that giving GNT to animals at a dose of 80 mg/kg body weight once daily for eight days caused a highly significant rise in MDA levels compared to control animals.

Oxidative stress is one of the primary mechanisms producing liver damage and the onset of sickness. In keeping with this earlier explanation, the current investigation found that the antioxidant enzymes GPx, SOD, and CAT activities considerably decreased in the gentamicin-administered group compared to the healthy control group. On the other hand, the level of lipid peroxidation was significantly increased, These findings are consistent with prior reports (Al-Kenanny *et al*., 2012; Ademiluyi *et al*., 2013; Mustafa *et al*., 2017). These findings could be used to explain how GNT increases oxidative stress, increases the formation of free radicals, and overwhelms the kidneys' antioxidant protective mechanism.

The GNT-induced aggravation of lipid peroxidation degrades membrane lipids and results in necrosis and damage. In addition to negatively affecting membrane lipids, GNT's suppressive action on enzymatic and non-enzymatic antioxidants causes an excess generation of reactive oxygen species, which also degrades proteins and nucleic acids. In turn, this results in renal toxicity, malfunction, and harm.

Following GNT-induced toxicity, the altered tissue scavenging capacity was recovered by oral administration of ALO 10 mg/kg b.wt. or LC 50 mg/kg b.wt. for 21 consecutive days. This was done by significantly reducing MDA levels and significantly elevating tissue CAT, SOD, and GPx activities. When compared to mice given with either ALO or LC alone, the DPP-4 inhibitor with LC produced better results. The outcomes were in line with those of other studies, which found that LC treatment reverses the sharp rise in MDA levels as well as the sizable drops in GSH and CAT activities in the hepato-renal tissues (Elkomy *et al*., 2020).

In a doxorubicin-induced toxicity rat model, the naturally occurring substance caused a considerable decrease in cardiac MDA levels combined with a noticeably elevated level of GSH (Mustafa *et al*., 2017). Additionally, in rats with acute renal failure brought on by myoglobinuria, LC can increase the antioxidant enzyme activity, including CAT and GSH, and decrease the MDA content in renal tissues (Aydogdu *et al*., 2006). By significantly lowering oxidative stress and inflammatory indicators, dipeptidyl pepetidase 4 inhibitors (DPP-4 inhibitors) demonstrated a renoprotective effect against renal damage in rats, demonstrating their antioxidant and anti-inflammatory actions (Glorie *et al*., 2012; Nuransoy *et al*., 2015; Chang *et al*., 2015).

Vildagliptin has also been shown to have cardio- and neuroprotective effects in animal models of myocardial and cerebral ischemia/reperfusion, respectively, as a result of its capacity to significantly lower oxidative stress, demonstrating its antioxidant activity. (Bayrami *et al*., 2018; El-Marasy *et al*., 2018).

Vildagliptin, a powerful DPP-4 inhibitor, has also been demonstrated to have renoprotective effects in renal damage following hepatic ischemia/reperfusion through decrease of oxidative stress and inflammation (Herzlinger and Horton, 2013). There are still many unknowns regarding the mechanisms underlying the renal protective effects of DPP-4 inhibitors, particularly ALO. The resolution of glucose toxicity and an improvement in blood glucose variability are a few potential explanations. (Del Prato, 2002). Renal protection may also be aided by improvements in the balance of body fluid volume brought about by ALO's encouragement of sodium excretion (Sherif *et al*., 2020).

Histopathological findings

Control negative rats (G1) had normal renal parenchyma and stroma with maintaining aspects of the nephron units, collecting tubules, papillary, and pelvic structures (Figs. 1, 2.).

Gentamicin-treated rats (G2) with renal serial sections showed considerable multifocal necrotic regions (coagulative necrosis) with pyknotic or karryoretic nuclei and deep eosinophilic cytoplasm but retaining a ghost of the tubular and glomerular architecture. Multiple distinct interstitial round cell aggregations, including lymphocytes, plasma cells, and macrophages, as well as varying degrees of degenerative changes, such as cloudy swelling and hydropic and vacuolar degeneration, as well as mild en-

largement of some distal convoluted and collecting tubules with partial atrophy of their lining epithelium, were observed. Hyaline intratubular casts occasionally appeared. A partial atrophy and shrinking of some glomeruli were seen. There may be mild to moderate perivascular oedema along with intertubular and glomerular blood vessel and capillary congestion (Figs 3, 4).

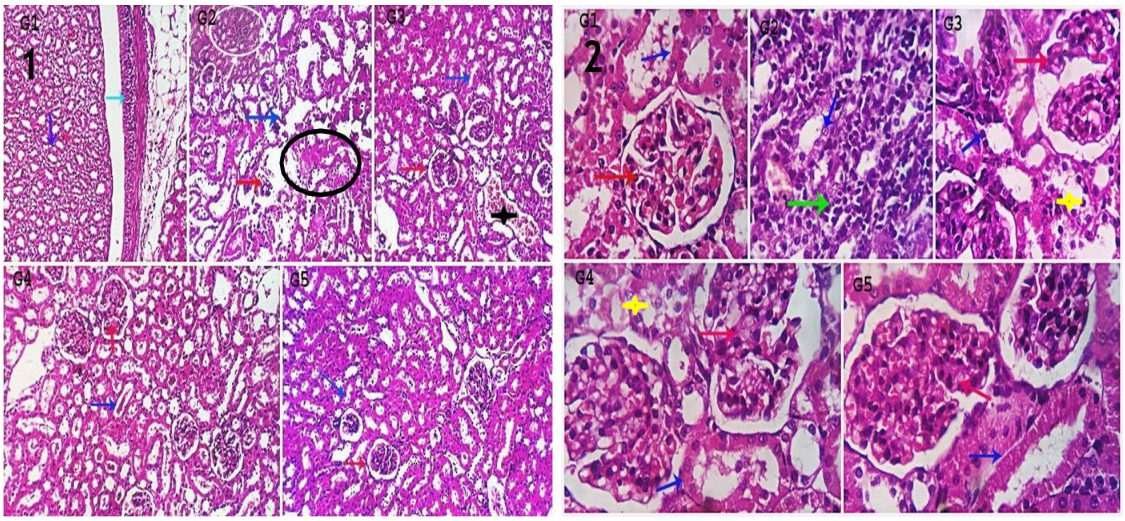
Sections from kidney of gentamicin treated rats followed by oral treatment of L-carnitin (G3), revealed apparently normal histo-morphology of nephron unites with keeping normal features of glomeruli, tubules, papillae, pelvis, vascular structures, and stroma. A few sections revealed mild congestion of renal blood vessels, degenerative changes (cloudy swelling and hydropic degeneration) and a few necrotic cells in some tubular epithelia beside dilatations in some distal convoluted and collecting tubules (Figs. 3, 4). kidney lesions of gentamicin treated rats followed by treatment with alogliptin (G4) were represented by mild perivascular edema and interstitial round cells infiltration.

While some nephron units (glomeruli and tubules) appeared to be normal, others displayed early necrotic and degenerative alterations, as well as sporadic cystic dilatation and intratubular hyaline casts in some tubules (Figs. 3, 4). Serial renal sections of rats treated with gentamicin and then given L-carnitine and alogliptin (G5) showed that the parenchymal and stromal structures appeared to be normal and had nephron unit keeping features, collecting tubules, papillary structures, and pelvic structures (Figs.1, 2).

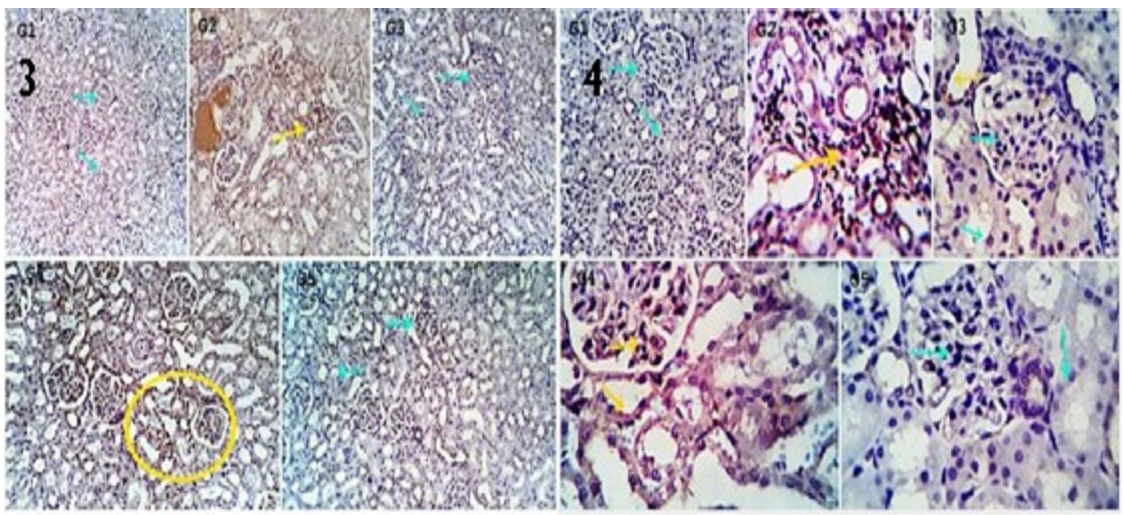
Immuno-histochemical findings

Immune-stained tissue sections from control negative rats (G1)) and gentamicin treated rats followed by treatment of L-Carnitine and alogliptin (G5) revealed negative nuclear and cytoplasmic immune -reactivity for the used inflammatory marker (NFKB).(Figs 3, 4) On the other hand moderate positive brownish cytoplasmic and nuclear staining reaction in almost all of the interstitially aggregated inflammatory cells could be observed in investigated immune-stained sections of gentamicin treated (G2) rats (Figs 3, 4).

Moreover, some of the degenerated tubular epithelial cells and glomerular endothelial cells, particularly at the vicinity of the aggregated inflammatory cells were also positively reactive. Gentamicin treated rats followed by L-Carnitine treatment (G3) showed a few reactive cells in some degenerated renal tubular epithelia (Figs. 3, 4). Immune-stained tissue sections from gentamicin treated rats followed by treatment of alogliptin (G4) showed a few positively expressed cells in the glomerular en-



Figs. 1, 2. Photomicrograph from rat's kidney of different experimental groups (1-5) showing apparently normal glomerular (red arrows) and tubular structures (blue arrows) in groups 1, 5. Tubular necrosis (Black circle), glomerular damage (red arrow) and interstitial round cells aggregation (Green arrow) are seen in G2. Vascular dilatation and focal tubular epithelial degeneration (Black and yellow stars) are seen in G3. Focal renal tubular degeneration, dilation and intratubular hyaline casts (Yellow star and blue arrow) are seen in G 4. H&E X 100, 200, 400.



Figs. 3, 4. Photomicrographs from rat's kidney of different experimental groups (1-5) immune stained by NFKB showing negative nuclear and cytoplasmic immune reactivity in groups 1, 5. (Light blue arrows) Characteristic positive immune reactivity (brownish cytoplasmic and nuclear staining) is seen in the interstitially aggregated round cells and in some tubular epithelia of G 2. (Orange arrows). A few reactive cells is seen in some degenerated renal tubular epithelia of G 3 (orange arrow). Some positively expressed cells can be observed in the glomerular endothelium and in degenerated tubular epithelia of G4. (Orange circle) . X 100, 400.

dothelium and in degenerated tubular epithelia (Figs 3, 4) The current study's histology and immunohistochemical clarifications are in rhythm and deeply anchored the biochemical and oxidant/ antioxidant features among the experimental animals.

The tubular and glomerular architecture could still be seen in the deep eosinophilic cytoplasm and moderate multifocal necrotic regions in the renal sections of GNT-treated animals. Round cell aggregations with many foci in the interstitial space were observed. It was shown that different degrees of degenerative changes, such as hazy swelling, vacuolar and hydropic degeneration, moderate dilatation of some distal convoluted and collecting tubules, and partial atrophy of their lining epithelium, existed.

The current investigation concentrated on the NFKB's immunohistochemistry localization in renal tissues, moderate positive brownish cytoplasmic and nuclear staining reaction in almost all of the interstitially aggregated inflammatory cells could be observed in investigated immune-stained sections of GNT treated rats.

Treatment with ALO or LC improved renal architecture noticeably, but the best outcomes were seen in rats treated with a combination of both substances because the immune-stained tissue sections showed negative nuclear and cytoplasmic immune reactivity for the used inflammatory marker (NFKB), and because the renal parenchyma and stroma were normal with maintaining features of the nephron units, collecting tubules, papillary, and pelvic structures. Normal renal corpuscles, renal tubules, and proximal, distal, and collecting (PCT, DCT, CT) convoluted tubules were visible in kidney slices from the LC group. (Elkomy *et al*., 2020).

Few studies have examined the beneficial effects of ALO on kidney histopathological and immunohistochemical traits. When researchers looked at the anti-inflammatory effects of ALO in a non-diabetic model of glomerular injury, they discovered that macrophage infiltration was reduced directly via the Glucagon-like peptide GLP-1-dependent pathway and that proteinuria in the rat Thy-1 nephritis model appeared to be improving.DPP-4 inhibitors' ability to control inflammation may have the potential to slow the evolution of non-DKDs. All things considered, it is expected that linagliptin and other DPP-4 inhibitors will have these advantages solely because to its ability to decrease blood sugar. This is because the renoprotective effects of DPP-4 inhibitors are widely known (Ademiluyi *et al*., 2013)

Additionally, in both diabetic and non-diabetic animal models of nephropathy, DPP-4 inhibitors showed pleiotropic renoprotective capabilities, particularly antifibrotic actions mediated by interaction with miR and integrins. Additionally, it appears likely that this class of drugs' antioxidant capabilities will have a conceptually distinct renoprotective impact. (Mustafa *et al*., 2017).

CONCLUSION

As demonstrated by the improvement of various biochemical indicators and oxidative state as well as the restoration of the renal structural integrity and function, treatment with alogliptin or L-carnitine protects the kidney through their antioxidant action. Better results are seen when the two are combined than each medicine alone.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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