

RESEARCH ARTICLE

Effect of Alogliptin and L-carnitine on Nephrotoxicity-Induced by Gentamicin in Rats

Sahar Sakr *and Mohamed Kamel

Pharmacology Department, Faculty of Veterinary Medicine, Zagazig University, 44511, Egypt

*Corresponding author e-mail: sakr84031@gmail.com

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Abstract

This research aims to investigate the nephro-protective effects of alogliptin and the supportive effects of L-carnitine in nephrotoxicity produced by gentamicin in male Wistar rats when coupled with a dipeptidyl peptidase-4 (DPP-4) inhibitor. Five equal groups (G) of 25 male albino rats, each weighing 130 ± 5.7 g, were created: G1 (control negative), G2 (control positive, nephrotoxic), G3 (L-carnitine-treated group), G4 (Alogliptin-treated group), and G5 (treated with alogliptin and L-carnitine). Significant variations were found in the biochemical analyses of the serum total protein, albumin, urea, uric acid, and creatinine. ($P < 0.05$) among groups, and the mean value of these parameters revealed that G5 was significantly different from G3 and G4. The concentration of malondialdehyde (MDA), glutathione peroxidase (GPx), catalase (CAT), and super oxide dismutase (SOD) were measured in order to determine the oxidative/antioxidant cascades. The mean value of these parameters revealed significant differences ($P < 0.05$) between various groups, with G5 being significantly different from G3 and G4. In gentamicin-induced nephrotoxicity models in rats, the findings of the histological and histochemical analyses suggest that alogliptin and L-carnitine may have a role in preventing the destruction of renal tissue. L-carnitine or alogliptin therapy appeared to preserve the kidney by its antioxidant effect as evidenced by the enhancement of biochemical indices, oxidant state, as well as the recovery of the kidney structural stability and its function. When the two medications are used together, the results are better than using each one separately. The improvement of physiological markers and antioxidant state, as well as the restoration of the kidney's structural integrity and function, demonstrate how administration of alogliptin or L-carnitine maintains the renal through their antioxidant effects. The results are better when the two drugs are taken together rather than individually.

Keywords: L: carnitine; DPP-4 inhibitor; Nephrotoxicity; Male Wistar rats; Gentamycin.

Introduction

The term "nephrotoxicity" refers to the toxic effects of drugs and other substances that cause a sharp decline in kidney function. Nephrotoxins are substances that cause kidney damage. Nephrotoxicity should not be confused with the need to adjust the dose of some medications for the reduction in renal function since these

medications are primarily excreted through the kidneys (e.g., heparin) [1].

A number of factors, causing renal toxicity, inflammation, damage of glomeruli, crystal nephropathy, and thrombotic events, can contribute to the complicated condition known as nephrotoxicity. Microbial angiopathy [2] using dipeptidyl peptidase IV inhibitors (DPP-4 inhibitors) a novel family of oral

hypoglycemic medications, increases the activity of endogenous incretin, which is a degrading enzyme of incretin (glucose-dependent insulinotropic polypeptide and glucagon-like peptide-1). DPP-4 inhibitors decrease glucagon production by increasing glucagon-like peptide-1. They are usually well tolerated due to the low risk of hypoglycemia and other side effects [3].

Alogliptin (ALO) benzoate, a novel DPP-4 inhibitor being evaluated in Japan for the type two diabetes treatment has been given clinical approval (T2DM) [2]. It was discovered that an alogliptin with a high DPP-4 selectivity was made using a structure-based methodology. Alogliptin's efficacy and safety have been compared to those of other DPP-4 inhibitors in recent clinical trials, which have shown that this is true. The focus of this updated review of a previous article is alogliptin's clinical efficacy and safety in the treatment of type II diabetes [4]. Seventy-five percent of its dietary sources are derived from endogenous biosynthesis, which occurs primarily in the liver and kidney [5, 6].

The process by which adenosine triphosphate ATP is the mitochondrial beta oxidation of fatty acids depends on leucine (LC). Therefore, LC can prevent the apoptosis that certain cell types' mitochondrial oxidative stress causes to their organelles [7]. Moreover, significant LC regulation of antioxidant processes was discussed in a number of organs, such as the colon, brain, retina, and heart [8-10]

L-carnitine is a water-soluble antioxidant primarily obtained through dietary sources in humans. About 25% of

the time, the body creates this amino acid from lysine and methionine [11]. It has been discovered that carnitine and its acyl derivatives may possess antioxidant properties [12]. Along with increased oxidative stress, carnitine also showed positive effects with regard to coronary artery disease, heart failure, and renal failure [13].

Levo-carnitine (L-carnitine) is an antioxidant supplement that is made from the naturally occurring amino acid derivative 3-hydroxy-trimethyl-aminobutyric acid. Endogenous production of L-carnitine takes place in the brain, kidneys, and liver [14]. L-carnitine is an essential component of energy production because it makes it easier for fatty acids to enter mitochondria, which raises mitochondrial activity [15]. The majority of L-carnitine metabolism occurs in the kidneys. After renal excretion, the majority of the L-carnitine is absorbed into the proximal tubules of the kidneys [16].

This investigation was conducted to examine the histological, antioxidant, and protective L-carnitine and alogliptin's effects on gentamicin administered albino rats with induced nephrotoxicity.

Material and Methods

Laboratory animals

For this study, the experiment was performed using 25 male white albino rats, each weighing 130 ± 5.7 g. The rats were obtained from Laboratory Animal Center, Faculty of Veterinary Medicine, Zagazig University, Egypt. They had a two-week conditioning period before the experiment.

The experimental design of the current investigation was authorized by the

ethical review committee for the Faculty of Veterinary Medicine at Zagazig University in Egypt (Approval No ZU IACUC/2/F/274/2022). All rats were given a standard, commercially balanced diet and unrestricted access to water.

Chemical compounds

- Gentamicin (Schering-Plough, which made the drug Garamycin® available in Egypt).

Each vial contains 80 mg, and it was administered via intraperitoneal injection (I/P).

- Alogliptin (Inhiglip®) was purchased from Kima, Egypt, and was administered orally once daily at a dosage of 10 mg/kg body mass.

- L-carnitine (Carnitol® 50 mg) was administered once daily via oral syringe at a dose of 50 mg/kg body weight which was acquired from Global in Egypt.

All equipment for biochemical analysis (serum total proteins and serum albumin) was obtained from the Bio Diagnostics Company (Dokki, Giza, Egypt).

Experimental design

Five equal groups of male albino rats were randomly assigned for the investigation (5 rats each). As a control, group I was assigned and received saline orally once daily for 21 days straight. Groups II, III, IV, and V received gentamycin (GNT) treatment [17] (80 mg of intraperitoneal medication per kg of body weight for ten consecutive days), but group II (control positive) was not given any medication for treatment of nephrotoxicity. Group III received oral L-carnitine (50 mg/kg body weight) for 21 non-stop days [18]. Group IV received an

oral dose of alogliptin (10 mg/kg body weight) once a day. Group V was given alogliptin (10 mg/kg body weight) and L-carnitine (50 mg/kg body weight) [19].

Sampling

Each rat underwent a puncture to obtain blood samples after the experiment had been completed for 24 hours. The blood samples were then held for 30 minutes at room temperature in a tilted position to promote blood coagulation before being centrifuged at 3000 RPM for 15 minutes to obtain serum to be used for biochemical analysis.

After blood collection, all of the animals were humanely decapitated, and their kidneys were removed. For histological and immunohistochemical studies, the kidney tissues were promptly fixed in 10% neutral buffered formalin for 48 hours. All rats (25), along with any additional samples, were buried in a carefully and hygienically prepared burial trench.

Serum biochemical studies

According to a previously published protocol [20, 21], serum total proteins and serum albumin were evaluated. Serum globulin was calculated as the sum of total proteins minus albumin [22]. In order to evaluate the kidney function, the serum levels of urea, uric acid, and creatinine were also determined [23, 24].

Oxidative/antioxidant cascade detection

By using specialized diagnostic kits provided by the Egyptian Bio Diagnostic Company in various experimental groups, the following measurements were made in order to identify the oxidative/antioxidant cascades: glutathione peroxidase (GPx)

[25], catalase (CAT) [26], and super oxide dismutase (SOD) [27] activities, and the concentration of malondialdehyde (MDA) [27].

Histological Analysis

Rat renal tissue samples that had been formalin-fixed were processed using an automated tissue processor. The process involved dehydration and a two-step initial fixation.

Following a 48-hour soak in 10% buffered formalin, the tissue is fixed by removing it from the fixative for 30 minutes with distilled water. After that, the tissues were dehydrated by being exposed to increasing concentrations of alcohol (70, 90, and 100%). First, the tissue was exposed to 70% alcohol for 120 minutes, then to 90% alcohol for 90 minutes, and finally, to 100% alcohol twice for one hour each. It involved submerging the tissue for 1.5 hours in pure xylene after submerging it for an hour in a mixture of 50% xylene and 50% alcohol. The samples were first immersed in molten paraffin wax, followed by blocking and embedding. Hematoxylin and eosin was used to stain the 4-5 um thick paraffin slices (HE) [28]. The tissues under evaluation were stained to detect any pathological changes, such as inflammatory conditions, circulatory troubles, degenerations, apoptosis, necrosis, and other changes.

Histo-chemical Analysis

Renal tissues from control free rats were found to be free from abnormal glomerular or tubular replacement by reticuline fibers, which were seen as a framework fixing functional tissue elements of the corresponding tissues. This information was revealed by tissue sections stained with the Gomori Silver

technique to declare the amount of reticuline fibres deposition.

Statistical analysis

Statistical Package for the Social Sciences (SPSS) was used to conduct a statistical analysis (Version 26, SPSS Inc, Chicago, IL, USA). The Duncan test was employed as a post hoc analysis after the one-way ANOVA to examine the differences within the groups which have been statistically significant. The results were presented as standard error of the mean (SEM) and the significance level was chosen at $P < 0.05$.

Results and Discussion

Serum Biochemical Analysis

This study investigated the possible renoprotective benefits of alogliptin and/or L-carnitine in a rat model of gentamicin-induced nephrotoxicity. Antibacterial therapy has historically used aminoglycoside gentamicin comes from *Micromonospora purpurea*, an antibiotic called an aminoglycoside. It is not effective against the majority of fatal Gram-negative bacterial infections [29].

The essential drug GNT is used to treat a variety of ailments in chickens and other animals, such as salmonellosis and colibacillosis [30]. Antibiotic adverse reactions are frequently brought on by one of 3 mechanisms: the medication's therapeutic effects being exaggerated, an immune system drug or its metabolites, or a response to the drug or its metabolites having hazardous effects.

In the current investigation, rats received 80 mg/kg of gentamicin intraperitoneally every day for 10 days straight. Increased serum levels of both creatinine and urea show that this

considerably affects renal function[31, 32].

The albumin and total protein percentages both dramatically decreased as compared to the control group. A rise in serum levels of urea and creatinine, as well as severe proximal tubules necrosis, which is eventually followed by deterioration and kidney failure, identified the complicated phenomena of nephrotoxicity caused by GNT (Table 1).

It is believed that reactive oxygen species are related to the toxicity of aminoglycosides, notably GNT [33].

Concurrent administration of LC improved the level of urea, uric acid, and serum creatinine that was brought on by GNT. The reduced levels of blood total proteins and albumin in rat treated with GNT were successfully recovered by the two medications. This increase in kidney function metrics can be attributed to the lowering of hematological distresses in the kidney brought on by GNT and increasing the antioxidant defiance effectiveness.

The results of the group treated with LC+GNT in this study are consistent with those reported previously [34, 5]. They demonstrated that LC lowers liver and kidney enzyme activity, oxidative stress, thioacetamide and tilmicosin damage from oxidative stress in rats. Histopathology and immunohistochemistry data backed up these findings. According to a previous study [35], MDA caused a considerable decline in the levels of the blood proteins albumin and globulin. ($P < 0.05$). The natural vitamin LC is required for the fatty acid oxidation process in the mitochondria to produce ATP [34, 36, 37]

Additionally, they promote β -oxidation, which lessens the negative effects of free fatty acids [38]. One of DPP-4 inhibitor given to persons with type 2 diabetes is analogliptin. The gathered data is consistent with their conclusions, according to the scientists [39] who examined the case of a 68-year-old male who had type II diabetes, hypertension, and cerebral infarction. ALO (25 mg/d) therapy reduced proteinuria, serum creatinine levels, and 2-microglobulin and -D-glucosaminidase excretion in the urine (NAG). However, there was a considerable decline in the excretion of albumin and total serum protein in the urine.

Oxidative stress evaluation in kidney

The activity of renal SOD was markedly decreased, but MDA was increased after 10 days of GNT treatment. Additionally, it was determined that SOD activity had decreased. Significantly, the same regimen of GNT administration caused a marked decrease in GPx activity ($P < 0.05$). Renal CAT, SOD, and GPx levels significantly increased, while MDA levels significantly decreased under therapy with LC and ALO (Table 2).

Rats administered GNT produced more superoxide anions as a result of increased H_2O_2 formation in the renal cortical mitochondria [40]. It is possible for H_2O_2 and superoxide anion to combine to create the unpredictable and reactive hydroxyl radical.

This radical is produced when Fe^{2+} is present. Therefore, Fe^{2+} appears to be essential for the production of reactive oxygen radicals associated with gentamicin nephrotoxicity. When oxygen radicals begin to accumulate, renal cells defend themselves by using various

antioxidant enzymes, such as GPx. Because of the direct toxicity of GNT or volume loss brought on by gentamicin therapy, together with a rise in lipid peroxidation by a reduction in the activity of antioxidant systems. A decrease in intracellular glutathione and an increase in H₂O₂ and hydroxyl radicals are the causes of GNT nephrotoxicity [41].

The aggravation of lipid peroxidation brought on by GNT leads to the degradation of membrane lipids, which also results in necrosis and damage[42, 43].Inhibition of both enzymatic and non-enzymatic antioxidants by GNT results in an excess production of reactive oxygen species, which not only damages membrane lipids but also degrades proteins and nucleic acids. As a result, renal toxicity, dysfunction, and damage occur.

The altered tissue scavenging capacity was restored following GNT-induced toxicity by giving an oral dose of LC 50 mg/kg body weight or ALO 10 mg/kg body weight for 21 straight days[44, 45]. By greatly increasing tissue CAT, SOD, and GPx activities and significantly lowering MDA levels, this was accomplished.

The findings were consistent with other studies that discovered that LC treatment reverses the significant drops in GSH, catalase as well as the sharp increases in level of MDA in the hepato-renal tissues [35, 46].

The naturally occurring substance markedly decreased cardiac MDA levels while also noticeably increasing GSH in a doxorubicin-induced toxicity rat model. LC can also increase the activity of antioxidant enzymes like catalase and GSH and decrease the cardiac MDA concentration in kidney in rats with myoglobinuria-induced acute renal failure[47-49]. The antioxidant and anti-inflammatory effects of PP-4 inhibitors were demonstrated by their ability to significantly reduce oxidative stress and inflammatory indicators, which protected against renal damage in rats[50-52].

Apotent DPP-4 inhibitor (Vildagliptin)has also been shown to reduce oxidative stress and inflammation, both of which have been discovered to have reno-protective effects on kidney damage brought on by hepatic ischemia/reperfusion [53]. Although it has been demonstrated that DPP-4 inhibitors, particularly ALO, have renal protective effects, the underlying mechanisms are still mostly understood.

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 on oxidative stress markers in kidney tissues in rats (n = 5). (Renal Malondialdehyde (MDA), Catalase(CAT), Glutathione Peroxidase (GPX), Super Oxide Dismutase (SOD) 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.

Data are expressed as the mean ± SE. Within each row, mean with different letters are significantly different at $p < 0.05$.

Histopathological findings

The normal renal parenchyma and stroma, nephron units, collecting tubules, papillary structures, and pelvic structures were still present in control negative rats (G1) (Figures 1-2). Although the tubular and glomerular architecture was still somewhat discernible in the renal serial sections of GNT-treated rats (G2), there were a substantial number of multifocal necrotic patches (coagulative necrosis)

with pyknotic or karyoretic nuclei and deep eosinophilic cytoplasm.

Lymphocytes, plasma cells, and macrophage were among the several interstitial round cell aggregations. Cloudy edoema, hydropic, and vacuolar degeneration were also observed, along with modest enlargement of some distal convoluted and collecting tubules and partial atrophy of their lining epithelium[54, 55].

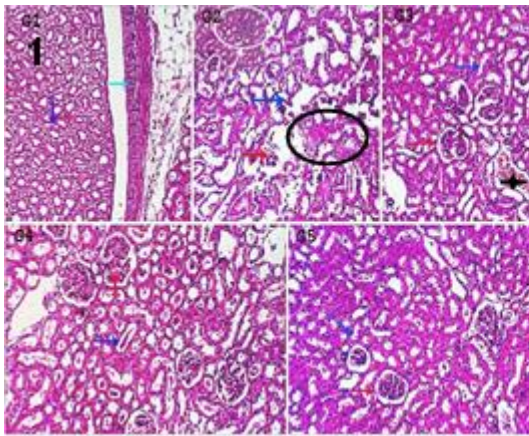
Occasionally, hyaline intratubular casts were visible. Some glomeruli showed a partial atrophy and some were getting smaller. There may be intertubular and glomerular blood vessel and capillary congestion in addition to mild to moderate perivascular oedema (Figures 1, 2).

Following GNT treatment in the kidney, rats given L-carnitin (G3) exhibited portions of the kidney that appeared to have the typical glomeruli, tubules, papillae, pelvis, circulatory networks, and stroma. Several sections also showed dilatations in certain distal convoluted and collecting tubules and a few necrotic cells in some tubular epithelia. in addition to a slight expansion of the renal blood vessels, hazy edoema, and hydropic degeneration (Figures 1, 2).

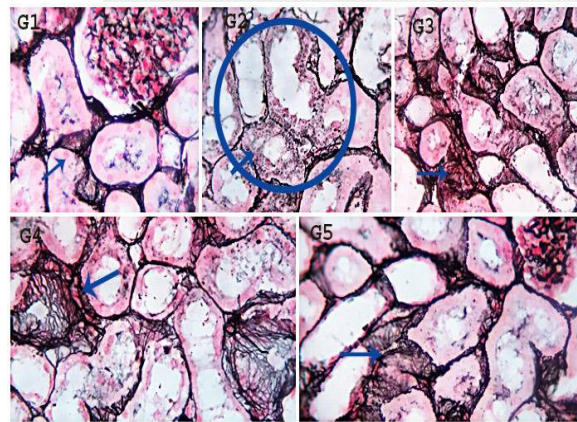
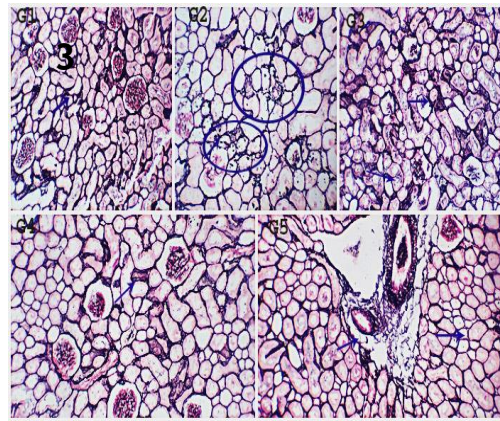
Mild perivascular edoema and interstitial round cell infiltration were present in the kidney lesions in the GNT- and alogliptin-treated animals (G4)[56].

The units of nephron (tubules and glomeruli) showed sporadic cystic dilatation, early necrotic and degenerative alterations, and intratubular hyaline casts in some tubules, while others appeared to be normal. (Figures 1,2). Serial renal sections of rats given L-carnitine and alogliptin (G5) after receiving gentamicin treatment revealed that the papillary structures, collecting tubules, and pelvic structures were present in the parenchyma and stromal structures and appeared to be normal (Figures 1,2)..

to declare the amount of reticuline fibers deposition, particularly those partially or completely replaced the renal necrotic glomerular and or tubular structures revealed that renal tissue of control free rats was free of abnormal glomerular or tubular replacement by reticuline fibers, which were seen as a framework fixing functional tissue elements of the corresponding tissues. On the other hand, necrotic and markedly degenerated renal tubules of gentamycin treated rats (G 2) showed abnormal deposition of tissue sections stained with Gomori silver technique reticuline fibers, partially or totally replacing reticuline fibers with minimal reticuline deposition around a few tubules or blood vessels in groups 3 and 5 respectively (Figures 3,4) [35].



Figures 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 and 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(left plate upper figures), 200 (left plate lower figures), 400 (right plate).



Figures 3, 4 showing a normal framework of supporting reticulin among renal parenchyma in G 1(blue arrow). Necrotic and markedly degenerated renal tubules of gentamycin treated rats (G 2) showing abnormal deposition of reticulin fibers, partially or totally replacing the necrotic tissues (blue circles and arrows). Experimental rats of groups 3, 4 and 5 showing a normal framework of supporting reticulin fibers with minimal reticulin deposition around a few tubules or blood vessels in groups 3 and 5 respectively (blue arrows). Gomori Silver stain X (100 Left plate), (400 right plate)

4

Conclusions

The improvement of physiological markers and antioxidant state, as well as the restoration of the kidney's structural integrity and function, demonstrate how administration of alogliptin or L-carnitine maintains the renal through their antioxidant effects. The results are better when the two drugs are taken together rather than individually.

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الملخص العربي

تأثير الألوجلبتين و ال- كارنتين على السمية الكلوية التي يسببها الجنتاميسين في الفئران

سحر صقر, محمد كامل

قسم العقاقير -كلية الطب البيطري- جامعة الزقازيق

تهدف هذه الدراسة الي فحص التأثيرات الداعمة للألوجلبتين و ال- كارنتين عند دمجهم مع مثبط DDP-4 والتأثيرات الوقائية للأوجلبتين في السمية الكلوية التي يسببها الجنتاميسين في ذكور الفئران. تمت هذه الدراسة على إجمالي عدد 25 من ذكور الجرذان البيضاء وزنها 130 ± 5.7 جم. وتنقسم إلى خمس مجموعات متساوية ، مجموعة 1: (كونترول سلبي) ، مجموعة 2: (كونترول إيجابي ، سمية كلوية) ، مجموعة 3: (المجموعة المعالجة ب ال- كارنتين) ، مجموعة 4: (معاملة بالأوجلبتين) و مجموعة 5: (معاملة بالأوجلبتين و L- كارنتين). في حين أن الجلوبيولين في الدم ليس معنوياً ، أظهرت النتائج البيوكيميائية للبروتينات الكلية والألبومين واليوريا وحمض البوليك والكرياتينين في الدم فروق معنوية ($P < 0.05$) بين المجموعات ، وأظهرت القيمة المتوسطة لهذه المعلمات أن مجموعة 5 كان مختلفاً بشكل كبير عن المجموعتين الثالثة والرابعة. تم قياس تركيز SOD ، CAT ، GPx ، MDA من أجل تحديد مضادات الأكسدة. أظهرت هذه المعلمات تغيرات معنوية ($P < 0.05$) بين المجموعات المختلفة ، وأشارت القيمة المتوسطة إلى أن مجموعة 5 كان مختلفاً بشكل كبير عن المجموعتين الثالثة والرابعة. في نماذج الفئران من السمية الكلوية التي يسببها الجنتاميسين ، تشير نتائج التحليلات النسيجية والكيميائية النسيجية إلى أن الأوجلبتين و ال- كارنتين قد يكون لهما دور في منع تدمير الأنسجة الكلوية. يحمي العلاج بالأوجلبتين أو ال- كارنتين الكلى من خلال عملهما كمضاد للأكسدة ، كما يتضح من تحسين المؤشرات البيوكيميائية المختلفة وحالة الأكسدة وكذلك استعادة السلامة الهيكلية الكلوية ووظيفتها. عندما يتم أخذ الاثنين معاً يُظهر تحسين العلامات الفسيولوجية وحالة مضادات الأكسدة ، بالإضافة إلى استعادة السلامة الهيكلية ووظيفة الكلى، يحافظ تناول الألوجلبتين أو إلكارنتين على الكلى من خلال آثارهما المضادة للأكسدة. تكون النتائج أفضل عندما يتم تناول العقارين معاً بدلاً من تناولهما بمفردهما.