



Role of Olive leaves Zinc Oxide Nanoparticles in Alleviating The Molecular and Histological Changes of Kidney in Female Goats-Induced by Gentamicin (Part III)

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A B S T R A C T

This study aimed to investigate the protective influence of olive leaf extract zinc oxide nanoparticles (OLEZnONPs) complex against gentamicin-induced kidney dysfunctions in goats. Twenty five adult female goats were randomly divided into five equal groups and treated as follows: control group (C) administered sterile distilled water (IM) for 10 days, group G administered 25 mg/kg BW gentamicin (IM) for 7 days, group Z administered 10 µg/kg BW of OLEZnONPs (IP) for 3 days, group GTZ administered 25 mg/kg BW gentamicin (IM) for 7 days and then 10 µg/kg BW of OLEZnONPs (IP) for 3 days, group GWZ administered 25 mg/kg BWs gentamicin (IM) and 10 µg/kg BW of OLEZnONPs (IP) together for first 3 days and then followed by gentamicin only for 4 days. After seven days of the experiment, the gene expression of kidney injury molecule-1 (KIM-1) and neutrophil gelatinase-associated lipocalin (NGAL) gene expression of kidney tissue were measured. In addition, samples of kidney were obtained for histopathological examination. Gentamicin medication induced a marked elevation in kidney tissue KIM-1 and NGAL gene expression in G and GTZ groups compared to control and other groups. Intra-peritoneal treatment of goats with OLEZnONPs did not significantly affect NGAL and KIM-1 gene expression in Z, GWZ, and control groups. Histologically, in contrast to control, gentamicin induced more extensive kidney damages such as necrotized glomeruli, atrophic glomeruli, and renal tubular epithelial necrosis, while it was found that these alterations in kidney tissues were improved in goats given OLEZnONPs with gentamicin compared to group G. In conclusion, our results demonstrate that OLEZnONPs reduce the deleterious effects of gentamicin with significantly decreasing of KIM-1 and NGAL gene expression and remodeling the histological changes of kidney in goats.

Keywords: olive leaves extract, OLEZnONPs, goats, kidney functions

INTRODUCTION

Aminoglycosides group includes several types of antibiotics, such as gentamycin which was

discovered in 1963. It is made from the bacteria *Micromonospora purpurea* (1, 2). Gentamycin is not metabolized to a measurable range and is fundamentally excreted from the body via the urinary excretion (3).

Several adverse effects of gentamycin can be ranged from minor to severe reactions such as, neurotoxicity (4, 5), neuromuscular problems (6) by reducing the efflux of calcium into presynaptic neuron and decreasing the acetylcholine amount which released into the synaptic cleft (7). In addition, gentamycin can cause ototoxicity (8, 9). About 53% of patients that were treated with gentamycin suffered from some type of kidney dysfunction (10). Many studies regarding nephrotoxicity induced by gentamycin were concentrated on the utilizing of different antioxidants such as hypoxia-inducible factor-1 α (11), vitamin C (12), N-acetylcysteine, vitamin E (13), and *Morinda citrifolia* extract (14).

Various molecular markers are being utilized to estimate the renal insufficiency as markers results from apoptosis and oxidative stress of cells of renal tubules involving KIM-1 (15, 16) and Neutrophil Gelatinase-Association Lipocalin (NGAL) (17, 18). Kidney injury molecule (KIM-1) is a transmembrane glycoprotein that is undetectable in routine techniques and is found on the apical membrane of proximal convoluted tubule following renal insufficiency and acute tubular necrosis, in addition to elevate the level of KIM-1 that is determined in urine in many kinds of nephrotoxicity (19-21). Whereas, NGAL (Neutrophil Gelatinase Association Lipocalin) is covalently attached to neutrophil-gelatinase of the lipocalin family. It is available in stomach, lungs, epithelial cells and colon situated in the proximal convoluted tubules and neutrophils (22). It is showed that NGAL may elevate in the thick ascending limb of the loop of Henle, distal tubule and collecting duct when destroyed (17). Therefore, the values of these biomarkers are used as early predictor of acute kidney impairment.

Zinc oxide nanoparticles (ZnONPs), particularly, are a friend to environment, fabricate easily, nontoxic, bio-safe and bio-compatible making them as a perfect candidate for biological applications (23, 24). According to available literatures, no detailed study is carried out to explore the effect of olive leave extract nanoparticles in modulating kidney dysfunction- induced by gentamycin in does. For the above reason, the present study was designed.

MATERIALS AND METHODS

Preparation of watery extract of olive leaves was done as described by (25) and synthesis of olive leaves extract of zinc oxide nanoparticles (OLEZnONPs) was prepared by dissolving zinc acetate dihydrate (0.25 g) in 50 mL deionized water. Then, 4 mL of the aqueous olive leaves extract (OLE) was added as drop by drop then the admixture stirred for 10 minutes using a magnetic stirrer. To adjust the pH of the solution to 12, NaOH (2 M) was added drop by drop during stirring till a white crystalline precipitate of zinc oxide was gained; then washed by distilled water and centrifuged (4000 rpm) for 5 minutes 3

times over and over by water and clarified by 70% ethanol. Finally, filtration and drying was done by an oven at 60 °C to gain the ZnONPs according to (26) and (27).

OLEZnONPs characterization was done by utilization of XRD, FTIR, and SEM techniques to reveal size, shape, crystallite, and functional groups with ZnONPs as mentioned by (28).

The effective dose (ED) of OLEZnONPs was 10 $\mu\text{g}/\text{kg}$ BW which was calculated after induction of acute kidney failure mediated by gentamycin (120 mg/kg BW). The researcher had a pilot study on Wistar albino strain female rats weighing 190 \pm 15 g and had age between 2-2.5 months using IM route technique. The effective dose of OLEZnONPs (10 $\mu\text{g}/\text{kg}$ BW) was applied on Iraqi local breed adult female goats of the current experiment.

Twenty goats (5-6 months age, weighing 20 \pm 3 kg) were randomly selected and divided into five equal groups. They were treated every day as follows: control group (C), administered sterile distilled water (IM) for 10 days; group G, administered gentamycin at 25 mg/kg BW (IM) for 7 days to induce acute renal insufficiency in goats; group Z, administered 10 $\mu\text{g}/\text{kg}$ BW (28) of OLEZnONPs (IP) for 3 days; group (GTZ): administered gentamycin 25 mg/kg BW (IM) for 7 days and then 10 $\mu\text{g}/\text{kg}$ BW of OLEZnONPs (IP) for 3 days; and group (GWZ), administered 25 mg/kg BW gentamycin (IM) and 10 $\mu\text{g}/\text{kg}$ BW of OLEZnONPs (IP) together for first 3 days then gentamycin only for the following 4 days.

Kidney specimens were collected from all groups of goats and kept in liquid nitrogen for measurement of KIM-1 and NGAL gene expression using PCR (by Bioneer kits, Korea). KIM-1 and NGAL gene primers obtained from gene bank data and designed. Total RNA was extracted from kidney of female goats by utilizing TRIzol® reagent kit and performed according to instructions of Bioneer company. The extracted total RNA was estimated and measured by Nanodrop spectrophotometer (THERMO, USA), There are two quality controls were performed on extracted RNA. The first one is to measure the quantity of RNA (ng/ μL). The second is to measure purity of RNA by absorbance reading in spectrophotometer at 260-280 nm in same Nanodrop machine.

The extracted total RNA was treated with DNase I enzyme to remove the trace amounts of genomic DNA from the eluted total RNA by utilizing (DNase I enzyme kit) and the above was done accordingly to method described by Promega company, USA instructions. DNase-I treatment total RNA samples were utilized in cDNA synthesis stage by utilizing AccuPower® RocktScript RT PreMix kit that provided from Bioneer company, Korea and done accordingly to company information. The RNA conveyed into cDNA in thermocycler under the thermocycler conditions. The qPCR master mix was done by utilizing AccuPower™ Green Star Real-Time PCR kit depend on SYBER Green dye determination of gene amplification in Real-Time PCR system. The results data of qRT-PCR for

studied and housekeeping genes were tested by the relative quantification gene expression levels (fold change) ΔCT Livak method described by (29). Furthermore, other samples of kidneys were immediately fixed in a 10% formalin solution for histopathological study according to (30).

Statistical analysis of data was done using SAS software (Statistical Analysis System, version 9.1). Data were analyzed as one-way analysis of variance (ANOVA). Means were separated using Fisher's least significant differences (LSD) at $P \leq 0.05$.

RESULTS

Figure 1(A and B) illustrates the mean values of KIM-1 and NGAL genes expression of the tissue of kidney in all treated groups. Treating adult female goats with a dose of 25 mg/kg BW gentamicin for 7 days led to high significant ($P < 0.05$) elevation in kidney tissue KIM-1 and NGAL gene expression compared to control and other treated groups. The values of KIM-1 and NGAL were 11.17 ± 1.8 , 1.14 ± 0.3 , 9.30 ± 1.05 , and 0.99 ± 0.22 for G and control groups, respectively. In addition, the results pointed a significant ($P < 0.05$) increase in gene expression of KIM-1 (4.01 ± 0.83) and NGAL (3.57 ± 0.29) in the GTZ group comparing to control group. Furthermore, non-significant ($P > 0.05$) varies were appeared in KIM-1 and NGAL gene expression between Z, GWZ, and control groups.

The microscopic examination of kidney samples revealed a normal histological structure in control female goats (Figure 2).

On the contrary, the microscopic examination of kidney sections of goats that were treated by gentamicin only, showed necrotized glomeruli, atrophic glomeruli and renal tubular epithelial necrosis, in addition to epithelial necrosis of cortical proximal tubules also appeared in this group. Moreover, high magnification revealed glomerular destruction and epithelial necrosis of proximal renal tubules but, the medullary renal tubules was normal in the same groups (Figure 3). Furthermore, the sections of kidneys in other groups (Z, GTZ, and GWZ) showed normal kidney architecture (Figures 4, 5, and 6) with no noticeable histological changes as a control group.

DISCUSSION

KIM-1 is a type 1 cell membrane glycoprotein that is rapidly produced, and the generation of protein occurs in high levels at the apical surface of the proximal tubule and its mRNA levels may be markedly increased after onset of renal insufficiency (19). The data achieved by (31) and (32) confirmed that KIM-1 is not as traditional markers (serum creatinine and BUN), The KIM-1 is qualified for the determination of acute nephrotoxicity which is induced by drugs in rats (5). KIM-1 is used as a biomarker for nephrotoxicity in preclinical studies of drugs (33). Furthermore, the Food and Drug Administration (FDA) has newly recognized KIM-1 as a suitable biomarker for renal insufficiency in preclinical studies of pharmacological agents.

It was found that NGAL is one of the most up-regulated

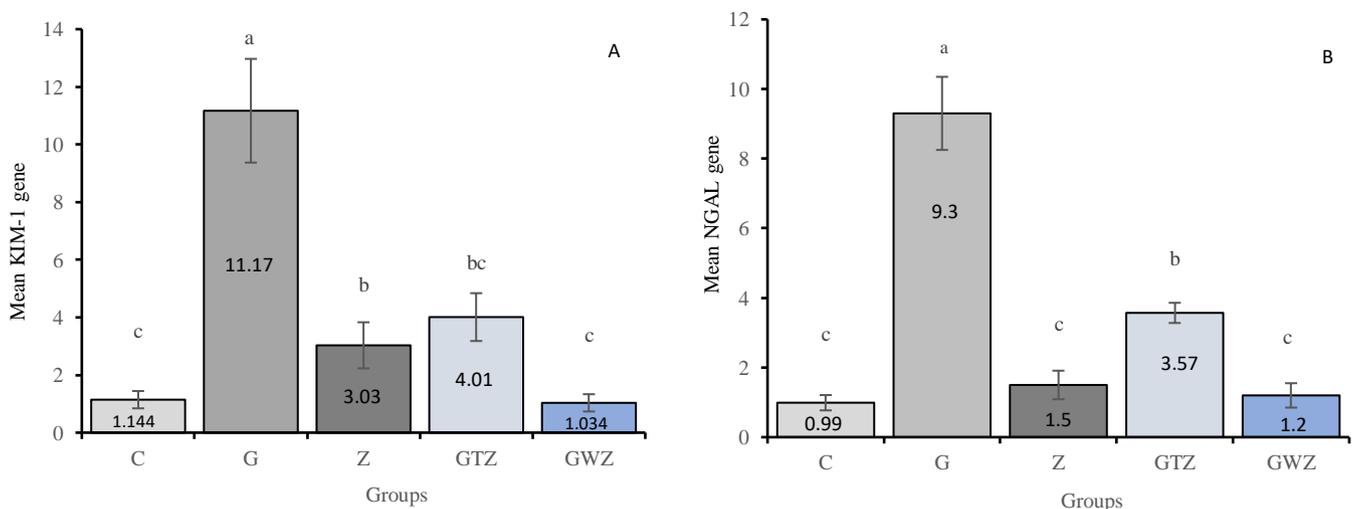


Figure 1. Shows (A) PCR KIM-1 (CT) and (B) NGAL (CT) gene expression of kidney tissue in experimental female goats. Values are expressed as means \pm SD (n = 5). C: control group received distilled water, G: group injected gentamicin (25 mg/kg BW IM) for 7 days, Z: Group injected ZnONPsOLE (10 $\mu\text{g}/\text{kg}$ BW IP) for 3 days, GTZ: grouped injected gentamicin (25 mg/kg BW IM) for 7 days and then followed by IP injection of ZnONPsOLE (10 $\mu\text{g}/\text{kg}$ BW) for following 3 days, GWZ: group injected gentamicin (25 mg/kg BW IM) and ZnONPsOLE (10 $\mu\text{g}/\text{kg}$ BW IP) for first 3 days of experiment and then gentamicin (25 mg/kg BW IM) only injected for following next 4 days. Means with different letters indicate significant differences ($P < 0.05$) between groupss

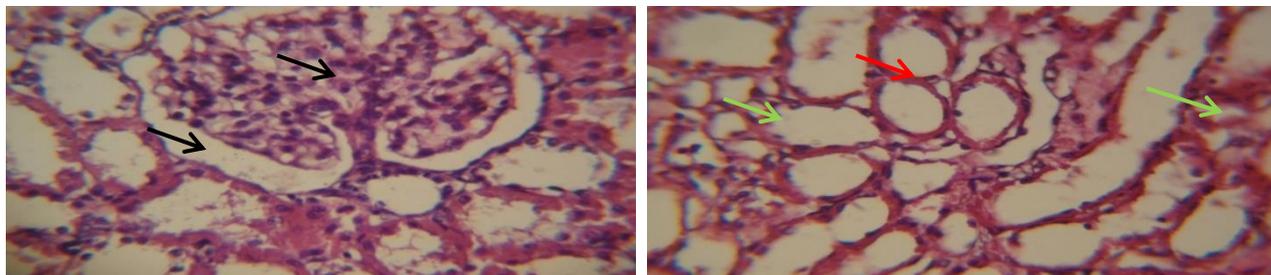


Figure 2. Histological section of kidney from control group shows normal renal medulla with normal renal tubules (black arrow) and normal renal cortex with normal glomerulus (red arrow) and renal tubules (green arrow). H&E 100× and 500×

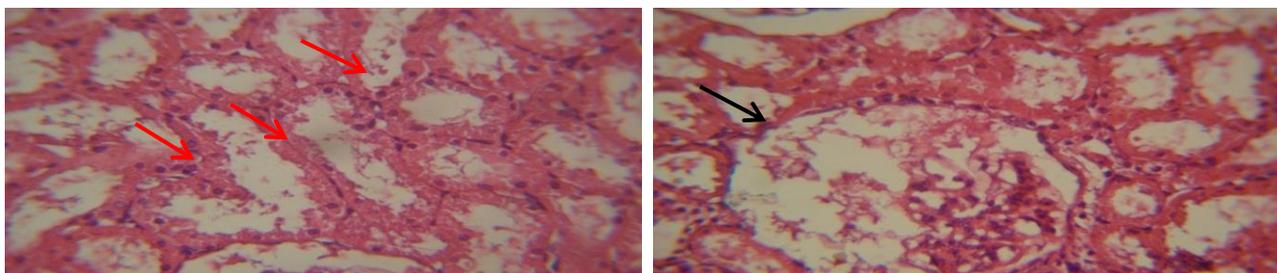


Figure 3. Histopathological section of kidney in gentamycin treated group shows glomerular necrosis (black arrow) and epithelial necrosis of proximal renal tubules in the cortex (red arrow). H&E 100× and 500×

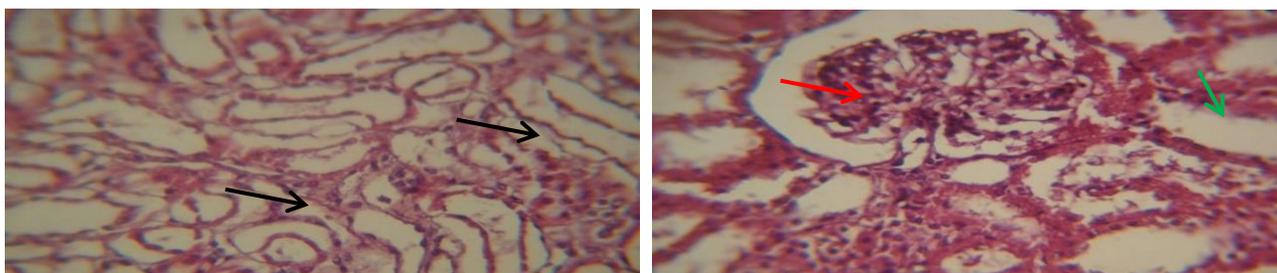


Figure 4. Section of kidney in ZNO group shows normal renal cortex with normal glomerulus (red arrow) and renal tubules (green arrow) and normal medulla with normal renal tubules (black arrow). H&E 100× and 500×

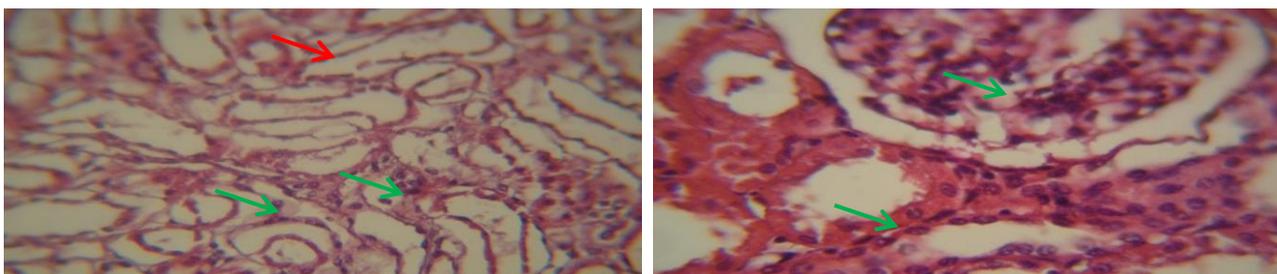


Figure 5. Section of kidney in GTZ group shows normal renal cortex with normal glomeruli (red arrow) and renal tubules (green arrow) and normal renal tubular epithelium in the medulla (green arrow). H&E 100× and 400×

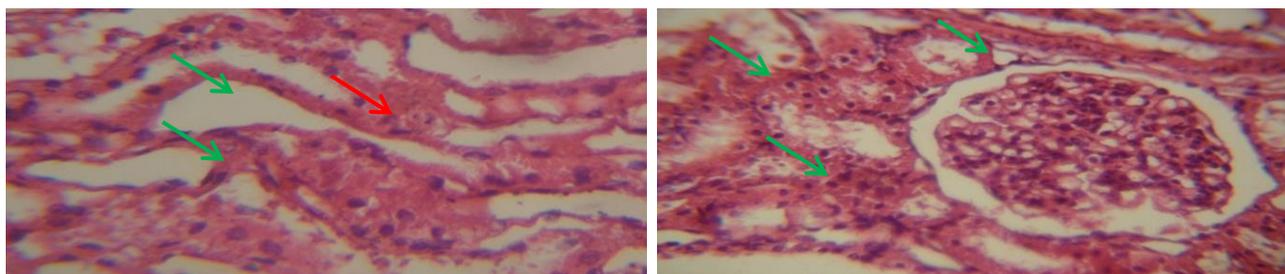


Figure 6. Section of kidney in GWZ group shows shows normal renal cortex with normal glomeruli (red arrow) and renal tubules (green arrow). H&E 100× and 400×

genes in the early post-ischemic mouse kidney (23, 34); and one of the earliest and most strongly produced proteins in the kidney after ischemic or nephrotoxic AKI in animal models (35, 36). Importantly, NGAL protein is facily determined in the blood and urine quickly after acute renal insufficiency in pre-clinical researches (17, 18). In study of (37), microarray analysis demonstrated that approximately 1000 genes were up-regulated after administration of gentamicin to a dog, Twenty genes of which is been formerly concerning to drug induced renal insufficiency in various canines, rodents and/or humans (38-40). In the present study, the gentamicin increases the expression of KIM-1 gene which is due to intensive immunoreactions of KIM-1 gene in glomerulus and tubules (41). Our results are in coincidence with (42, 37, 43) studies in which the intensive immunoreaction of KIM-1 was reported after administration gentamicin.

In a previous study, the expression of KIM-1 in kidney tissues was measured to evaluate the role of an antioxidant in acute kidney injury (AKI) mice, KIM-1 was decreased to normal range in the treated group, near to the range showed in healthy mice (44). Roudkenar and his colleagues indicated that NGAL has cytoprotective influence and may protect cells against oxidative stress (45). The information also robustly supposed that induction of NGAL at hurtful conditions is a compensatory response to improve oxidative stress intermediated toxicity. Zhao and his colleagues demonstrated that using certain antioxidant (Self-assembling peptide-Mito-2,2,6,6 tetramethylpiperidine-N-oxyl) significantly decreased acute kidney lesions involving tubular necrosis, apoptosis and lowering up-regulation of Kim-1 in ischemia-reperfusion injury mice, in addition to exerting better kidney protective effect on acute kidney insufficiency (46).

The results of the current study revealed variable pathological alteration in renal system of goats treated with gentamicin, indicating nephrotoxicity. Histological changes accompanied by gentamicin treatment vary from renal tubular epithelium cell necrosis, necrotizing glomeruli and atrophic of glomeruli. These results showed agreement with (47-49). Researchers have shown that gentamicin caused apoptosis in glomeruli and tubules in which gentamicin led to a significantly increased in the amount of cleaved caspase 3, Bax, and p53 protein expression while it decreased the Bcl-2 protein expression (50). Epithelial necrosis of proximal renal tubules in the present study demonstrated that administration of gentamicin may lead to significant alterations in renal tubules that may be refer to reabsorption of gentamicin in proximal convoluted tubules; in addition to the degeneration and necrosis of the epithelial cells of the tubules as mentioned by (12, 41). Furthermore it was manifested by dilated tubules, loss of brush border, severe leucocytic infiltrations, tubular degeneration and presence of tubular casts. In present

study, the groups are treated with ZnONPsOLE, after and with gentamicin treatment showed on histological changes and this finding agreed with (51) who demonstrated that ultrastructural analysis of the kidney showed no marked pathological alterations, even though the atomic absorption data indicated a substantial bioaccumulation of Zn in this organ.

(52) and (53) demonstrated that severity of the desquamation of tubular lining epithelia led to cellular casts' formation associated with increasing in the dose of ZnO-NPs, nephrotoxicity disruption in the energy metabolism with subsequent mitochondrial dysfunction with impairment of the cell membrane, ending by necrosis of tubular epithelial cell lining (54). In the present study, the 10 µg/kg BW of OLEZnONPs is very low dose. The results found by Teslariu and his colleagues (55) agreed with our study in which the renal injuries induced by gentamicin can be reduced by Zn supplementation, while, there is minor intense injuries, mild degree of tubular necrosis, moderate desquamation of basement membrane cells and major of cells preserving the cellular membrane. The tubular regeneration was also presented with a medium intensity, and the inflammatory processes were in the 1st degree of severity compared to gentamicin group. In conclusions, the current study demonstrated that gentamicin potentiated renal dysfunction and significantly increased gene expression of KIM-1 and NGAL. Moreover, it was found that OLEZONPs alleviating the deleterious effects of gentamicin with remodeling the histological changes of kidney in does.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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دور أوراق الزيتون النانوية في جزيئات أكسيد الزنك في التخفيف من التغيرات الجزيئية والنسجية في الكلى عند الماعز التي يسببها الجنتاميسين (الجزء الثالث)

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هدفت هذه الدراسة إلى دراسة الآثار الوقائية لمركب جزيئات أكسيد الزنك (OLEZnONPs) المستخرج من أوراق الزيتون ضد اختلالات وظائف الكلى التي يسببها الجنتاميسين. تم تقسيم 25 من الماعز البالغين بشكل عشوائي إلى خمس مجموعات متساوية وعولجوا يوميًا على النحو التالي: تلقت المجموعة السيطرة (C) ماءً مقطر (عضلياً) لمدة 10 أيام، تلقت المجموعة (G) 25 مغ / كغ من وزن الجسم. الجنتاميسين (عضلياً) لمدة 7 أيام، تلقت المجموعة (Z) 10 مايكرو غرام / كغ من وزن الجسم. من (OLEZnONPs) (داخل البيريتون) لمدة 3 أيام، وتلقت المجموعة (GTZ) 25 مغ / كغ من وزن الجسم. الجنتاميسين (عضلياً) لمدة 7 أيام، ثم 10 مايكرو غرام / كغ من وزن الجسم من (OLEZnONPs) (داخل البيريتون) لمدة 3 أيام، وتلقت المجموعة (GWZ 25) 25 مل/كغ، وزن الجسم جنتاميسين (عضلياً) و 10 مايكرو غرام من (OLEZnONPs) (داخل البيريتون) معاً لمدة 3 أيام الأولى ثم يتبعها معاملة بالجنتاميسين لمدة 4 أيام فقط. في نهاية تجربة التعبير الجيني لـ KIM-1 و NGAL في نسيج الكلى تم قياسهما. بالإضافة إلى ذلك، تم الحصول على عينات من الكلى لغرض الدراسة النسيجية. تسببت الجرعة الزائدة من الجنتاميسين في زيادة معنوية في تعبير أنسجة الكلى للجيني KIM-1 و NGAL في مجموعات (G) و (GTZ) مقارنة بمجموعة السيطرة والمجموعات الأخرى المعالجة. علاج الماعز بواسطة OLEZnONPs تسبب باختلافات غير معنوية لوحظ في التعبير الجيني لكل من المجموعتين (Z، GWZ) ومجموعات السيطرة، وهذا قد يكون بسبب الآثار الوقائية من OLEZnONPs. على النقيض من السيطرة، تسبب الجنتاميسين في حدوث أضرار أكبر في الكلى مثل تنخر الكبيبات و ضمور الكبيبات ونخر الظهارة الكلوية الأنبوبية، حيث تبين أن هذه التعديلات في أنسجة الكلى ستؤدي إلى تحسن في الماعز التي تلقت OLEZnONPs مع الجنتاميسين (G). في الختام، أشارت نتائجنا إلى أن OLEZnONPs يخفف من الآثار الضارة للجنتاميسين مع انخفاض ملحوظ في التعبير الجيني لـ KIM-1 و NGAL ويعيد تشكيل التغيرات النسيجية المتضررة للكلى.

الكلمات المفتاحية: مستخلص أوراق الزيتون، OLEZnONPs، ماعز، وظائف الكلى