Protective Effect of *Terfezia claveryi* Extract on Gentamicin-Induced oxidative stress in Rats

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Abstract
Gentamicin is one of the commonly use antibiotic for gram negative spectrum, and this drug is associated with nephropathy this due to the formation of reactive oxygen species. The aim of this study to evaluate the ability of *Terfezia claveryi* extract for treatment the oxidative stress induced by using gentamicin. To reach our object 24 rats were divided in to four groups all of them received water and standard diet while the treatment of the control groups were placebo treatments, the Gentamicin group received the drug with a ratio of 100mg/kg for 8 days, *Terfezia claveryi* group received oral dose of extract with a ratio of 250 mg/kg for the same period, the last group which is Gentamicin *Terfezia claveryi*; received drug with a ratio of 100mg/kg and in the same time received oral dose of extract with a ratio of 250 mg/kg for 8 days. The results show significantly increase in serum urea and creatinine which is associated with histopathological damage of the kidney tissue's architecture due to the administration of the gentamicin and this increase or damage is positively correlate with concentrations of the kidney MDA and negative correlation with the activity of catalase, superoxide dismutase and glutathione peroxidase on the kidney tissue homogenate, this modulation of the biological parameter histological damage is significantly neutralized by the administration of the *Terfezia claveryi* extract, from which we can conduct that administer of the *Terfezia claveryi* extract reduce the toxicity and damage caused by the gentamicin.

الاثر الحافظ لمستخلص الكمأ الصحراوي على الجهد المؤكسد الناتج من الجنتامايسين في الجرذان

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الملخص
الجنتامايسين هو احد المضادات الحيوية المستخدمه للطيف السالب من الجراثيم و هذا المضاد يكون استخدامه متزامن مع الاعراض المرضية للكلية بسبب قابلية هذا المضاد على تكوين مركبات الاوكسجين الفعالة. الهدف من هذه الدراسة تقييم استخدام مستخلص الكمأ الصحراوي لمعالجة جهد الاكسدة الناتج من استخدام الجنتامايسين. للوصول الى هدف التجربة تم استخدام 24 جرذ قسمت الى اربعة مجاميع حيث كانت تزود بالغذاء المعياري و الماء، استقبلت المجموعة الضابطة العلاج الكاذب، مجموعة الجنتامايسين حقنة بالجنتامايسين بتركيز 100 ملغ / كغم يوميا لمدة 8 ايام ، مجموعة مستخلص الكمأ اعطيت المستخلص بواسطة الانبوب المعوي بتركيز 250 ملغ / كغم لنفس الفترة الزمنية ، مجموعة الجنتامايسين-الكمأ حقنة بالجنتامايسين بنسبة 100 ملغ / كغم و اعطيت المستخلص بواسطة الانبوب المعوي بتركيز 250 ملغ / كغم لمدة 8 ايام. اظهرت النتائج زيادة معنوية في تركيز اليوريا و الكرياتنين في الدم و هذه الزيادة متزامنه مع التلف في معمارية خلايا الكلى نتيجة الحقن بالجنتامايسين و هذه الزيادة او التلف ترتبط ايجابيا بتركيز من المالونداي الدهايد وسلبيا بفعالية كل من الكاتاليز ، سوبراوكساید دسميوتاز و الكلوتاثايون بيروكسديز في خلايا الكلى وهذا الاختلاف في هذه التحاليل الحيوية عدلت باستخدام مستخلص الكمأ الصحراوي بصورة معنوية و من هذه النتائج نستنتج ان استخدام مستخلص الكمأ الصحراوي يقلل من التلف الحاصل من سمية استخدام الجنتامايسين.

Introduction

Oxidative stress is defined as the imbalance between reactive oxidants and antioxidants; it is produced when the reactive oxygen species are more than the antioxidant capacity or one or more of the antioxidant parameters are decreased or depleted (1, 2). Several conditions are associated with oxidative stress shortage of antioxidant vitamin and selenium intake, pollution, smoking, diseases, and drugs(3).

Gentamicin is an aminoglycoside antibiotic routinely used in every day clinical practice for the treatment of Gram-negative infections alone or in synergy with beta-lactam antibiotics. In addition to clinical effectiveness, a low rate of resistance and reasonable cost recommend gentamicin as the first line antibiotic for many severe and life-threatening diseases. However, its frequent use is limited by a risk of serious side-effects such as nephrotoxicity and ototoxicity. Incidence rates of gentamicin nephrotoxicity reported in some studies vary between 8% and 26% (4-6).

Most remarkable toxicities of gentamicin include ototoxicity and nephrotoxicity, nephrotoxicity occurs as a result of proximal tubular damage and glomerular dysfunction, it is because of gentamycin ability to stimulate the generation of reactive oxygen species (7) which enhanced lipid peroxidation (8-12)

Terfezia claveryi grow naturally in many parts of the world including particular localities of Arabian Desert (13), and traditionally used in folk medicine for the treatment of eye ailments in Iraq, Saudi Arabia and the Eastern Badia of Jordan. it is documented that *Terfezia claveryi* have antimicrobial properties ageist *Staphylococcus aureus* (14) and hepatoprotective effect against carbon tetrachloride toxicity (15), furthermore it was recorded that it has high content of antioxidants such as vitamin A, C B-carotene and many phenolic compounds that play an important role scavenger of reactive oxygen species as well as chelate ferric ions that inhabit lipid peroxidation which is the cell membrane damage caused by oxidative stress (16, 17).

Since *Terfezia claveryi* are very rich source of antioxidants and Gentamicin nephron toxicity majorly due to the oxidative stress. The present study was undertaken to evaluate the protective effect of *Terfezia claveryi* extract on Gentamicin induced oxidative stress.

Materials and methods

Chemicals

Gentamicin was obtained from local pharmacy; Urea and creatinine kits were purchased from randox®. SOD, CAT, and GPX kits were purchased from bio-vision®. lipid peroxidation kit were purchased from cell biolabs®.

Fungus material

Terfezia claveryi purchased from local markets of Baghdad, and authenticated by Professor Adel Al-Kenani from Baghdad University the sample was washed carefully, peeled and preceived at -20°C until use.

Tested animals

Twenty four adult male Wistar rats, weighing 150–190 g were used in the present study. Animals were housed in a central facility under controlled conditions (12 h light schedule, room temperature at 20 ± 2 C°). Feed and water were provided *ad libitum*, and acclimatized for 7 days before experimentation.

Extract preparation

Frozen samples of *Terfezia claveryi* were homogenized 1:3 (w/v) in cold distilled water using a household blender on full speed for 1 min. The homogenates were refrigerated overnight, then filtered through cheesecloth. The filtrates were centrifuged at 4000 rpm for 15 min. the supernatants were then dried using rotary

evaporator, the dried matter were re-suspended using distilled water and kept at -20°C until use (18).

Experimental protocols

Rats were divided randomly into four equal groups including six animals of each and all of them received water and standard diet:

- Control group (CON) the rats received a daily intraperitoneal (i.p.) injection of normal saline with a ratio of 100mg/kg/day and tab water via gavage with a ratio of 250mg/kg for 8 days.
- Gentamicin group (GEN) the rats were injected (i.p.) with 100mg/kg/day for 8 days (19) and tab water via gavage with a ratio of 250mg/kg for the same period.
- *Terfezia claveryi* group (TRF) rats in this group received extract via gavage with a ratio of 250mg/kg (20) and a daily intraperitoneal (i.p.) injection of normal saline in a ratio of 100mg/kg/day for 8 days.
- Gentamicin *Terfezia claveryi* group (GEN & TRF) the rats were injected (i.p.) with 100mg/kg/day and received *Terfezia claveryi* extract with total sold percentage of for 8 days.

Determination of experimental parameters

After 24 hours from the last treatment, blood were drawn from all rats by heart pincher method to determination of the renal function tests, and the kidney is collected for assay the malondialdehyde (MDA) concentration and the activity of enzymatic antioxidant system in the tissue, and for histopathological study.

Serum parameters

Serum urea assay

For the determination of serum urea we used the method of Barker (21), Diacetyl monoxime is hydrolyzed under acidic conditions to produce diacetyl which then condenses with urea to form a pink chromogen witches is enhancing the color development by using Thiosemicarbazide and ferric ions and this chromogen is measured at 520 nm.

Serum Creatinine assay

Determination of serum creatinine was done according to the Jaffe's method (22), Creatinine in serum directly reacts with alkaline picrate resulting in the formation of a red color, which is measured at 505 nm. Protein interference is eliminated using sodium lauryl sulphate.

Kidney tissue parameters

Preparation of the kidney homogenate

Rats were sacrificed the kidney was removed immediately, washed with ice-cold 0.15 M phosphate buffer saline pH 7.4, a portion of ten percent of kidney was homogenate with 1.15% KCl, the mixture centrifuged at 14000 rpm for 15 min and supernatant was used for measuring the parameters.

Determination of Malondialdehyde in kidney homogenate

Malondialdehyde was assayed according to Uchiyama and Mihara (23). MDA reacts in acidic media with Thiobarbituric acid (TBA) to produce pink colored complex, which is measured spectrophotometically at 535 nm.

Determination of enzymatic antioxidant parameters in kidney homogenate

Total superoxide dismutase (SOD)

SOD activity was measured according to the method of Winterbourn (24). It is based on the ability of superoxide dismutase to inhibit the reduction of nitroblutetrazolum (NBT) by superoxide, absorbances were monitored at wave length 560 nm.

Catalase (CAT)

Renal catalase was assayed according to the method of Beers and Sizer (25). Catalase catalyses the decomposition of hydrogen peroxide (H_2O_2) to water and oxygen, The enzyme activity was followed by the decreasing in absorbance at 240 nm at 15 second intervals.

Glutathione Peroxidase (GPX)

Renal glutathione Peroxidase was assayed according to the method of Leopold Flone etal (26). Oxidized glutathione formed during glutathione peroxidase reaction is instantly and continuously reduced by an excess of glutathion reductase activity for a constant level of glutathione. The concomitant oxidization of NADPH is monitored spectrophotometrically at 340 nm.

Estimation of Protein in the supernatant of liver homogenate

The total protein was estimated according to the method of Lowery et al (27), Using bovine serum albumin as standard.

Histological procedure

Small portion of kidneys cortex were fixed in 10% formalin, dehydrate in graded alcohol and embedded in paraffin wax, sectioned at 5 µm thickness and stained with Hematoxylin and Eosin for light microscopic examination (28).

Statistical analysis

The data were expressed as means ± standard errors (SEM). Differences between group means were estimated using a one-way analysis of variance (ANOVA) and the Tukey's test was done for multiple comparisons using the SPSS 12.0 for Windows. Results were considered statistically significant at $p<0.05$.

Results

The administration of gentamicin to the rats, cause the significant increase in blood urea, serum creatinine when we compared with control group (Figure 1, Figure 2). Co-administration of *Terfezia claveryi* extract will decrease the rise in blood urea and serum creatinine.

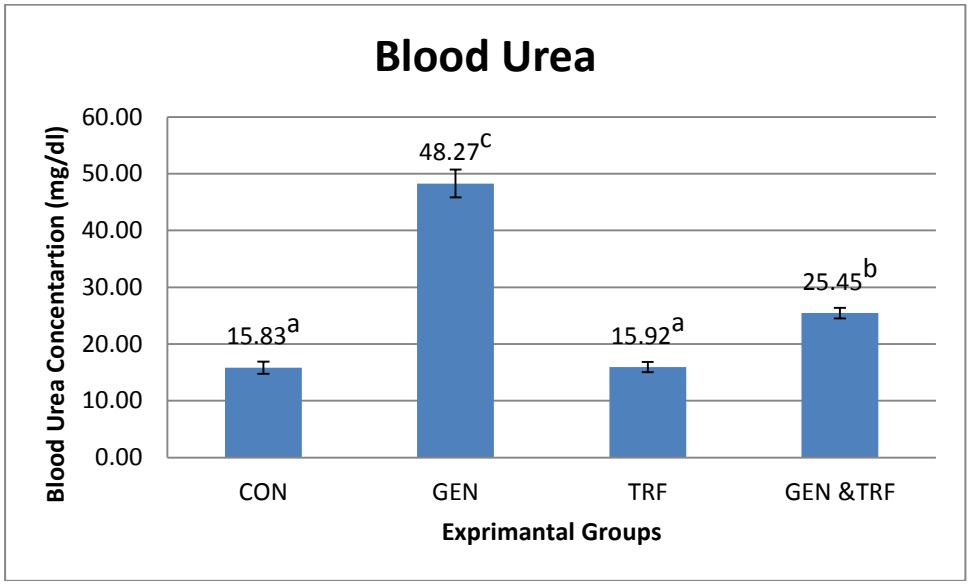

Figure 1 The effect of the treatments of the groups in blood urea concentration, values are expressed as mean ± SME (n=6) P values where calculated by student t-tests, mean with different superscripts (a, b, c) differ significantly, $P<0.05$

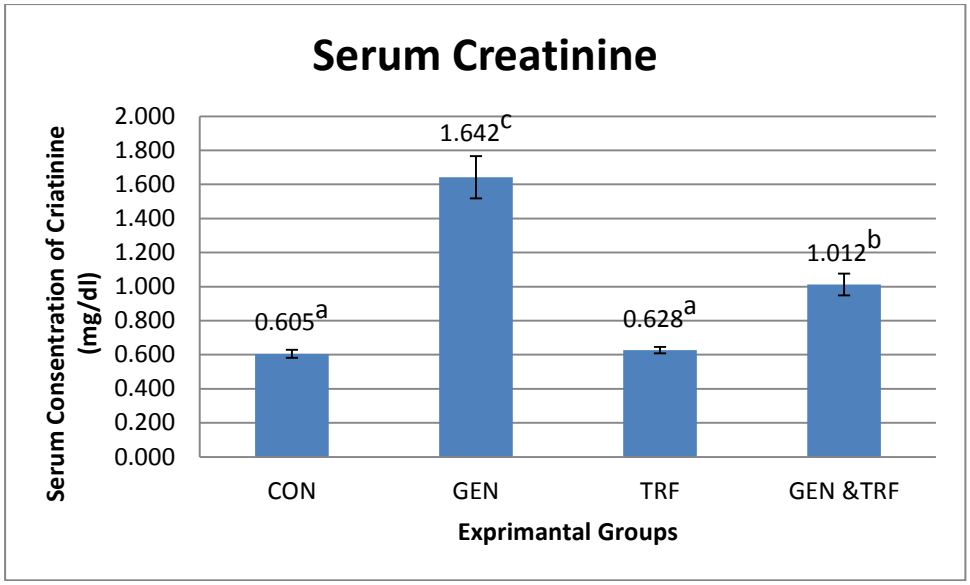

Figure 2 The effect of the treatments of the groups in serum creatinine concentration, values are expressed as mean ± SME (n=6) P values where calculated by student t-tests, mean with different superscripts (a, b, c) differ significantly, P<0.05

MDA was significantly increased in gentamicin treated group and this rise in MDA was decreased by *Terfezia claveryi* extract (Figure 3).

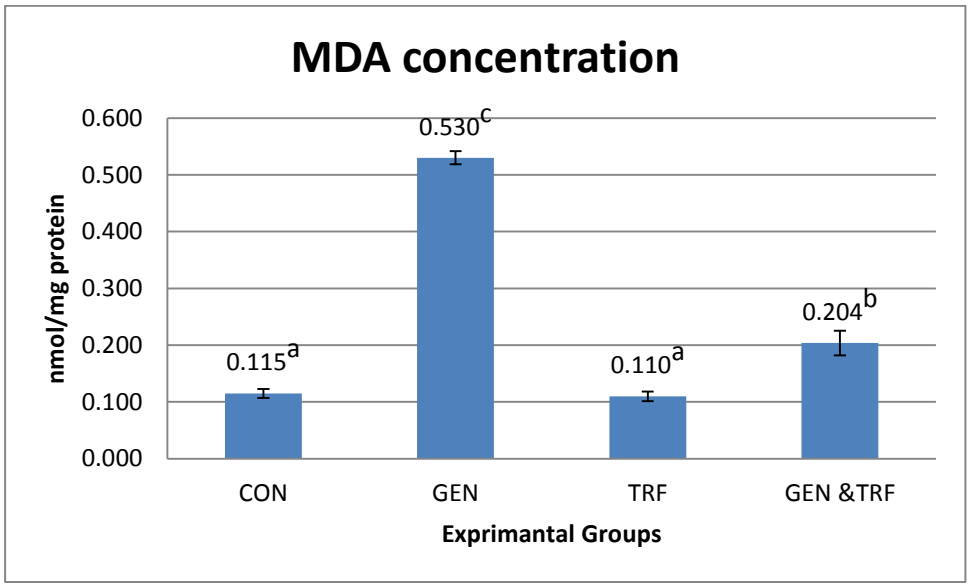

Figure 3 The effect of the treatments of the groups in MDA concentration in liver homogenate, values are expressed as mean ± SME (n=6) P values where calculated by student t-tests, mean with different superscripts (a, b, c) differ significantly, P<0.05

In the kidney tissues the enzymatic antioxidant activity shows that in the gentamicin-treated rats there was a significant decrease in the activity of SOD, GPX and CAT enzymes as compared to control group. However, administration of *Terfezia claveryi* extract to the Gentamicin-intoxicated rats significantly restored these parameters in the kidney tissue as we show in (Table 1).

Table 1 Show the antioxidant enzymes parameters in hepatic tissue

Experimental Groups	SOD	CAT	GPX
CON	9.26 ± 0.10[c]	0.929 ± 0.017[c]	2.218 ± 0.278[c]
GEN	3.20 ± 0.17[a]	0.320 ± 0.024[a]	0.557 ± 0.026[a]
TRF	9.32 ± 0.14[c]	0.927 ± 0.010[c]	2.318 ± 0.143[c]
GEN &TRF	7.18 ± 0.46[b]	0.737 ± 0.011[b]	1.267 ± 0.049[b]

Values are expressed as mean ± SME (n=6) P values where calculated by student t-tests, mean with different superscripts (a, b, c) differ significantly, $P<0.05$

Histopathological examination shows no changes of glomular structure of kidney tissue of the control rat when we compare it with kidney of TRF group (figure 4) and (figure 6) , on the other hand the administration of gentamicin alone cause necrosis and degeneration of glomerulus (figure 5) and this defects was decreased when we administrate the *Terfezia claveryi* extract (figure 7).

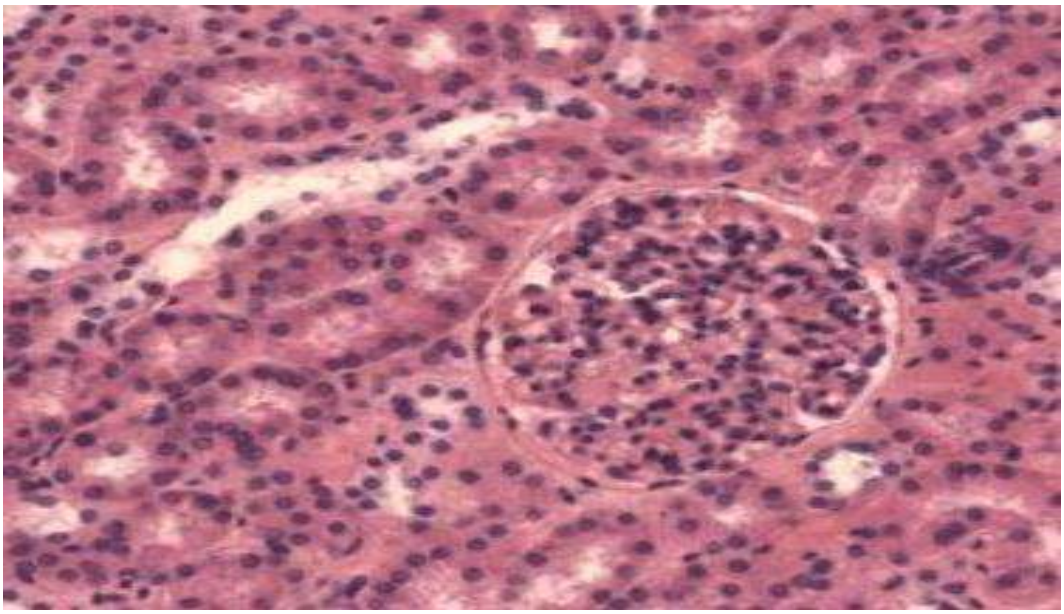

Figure 4 Kidney section from control group showing the normal structure of glomerulus

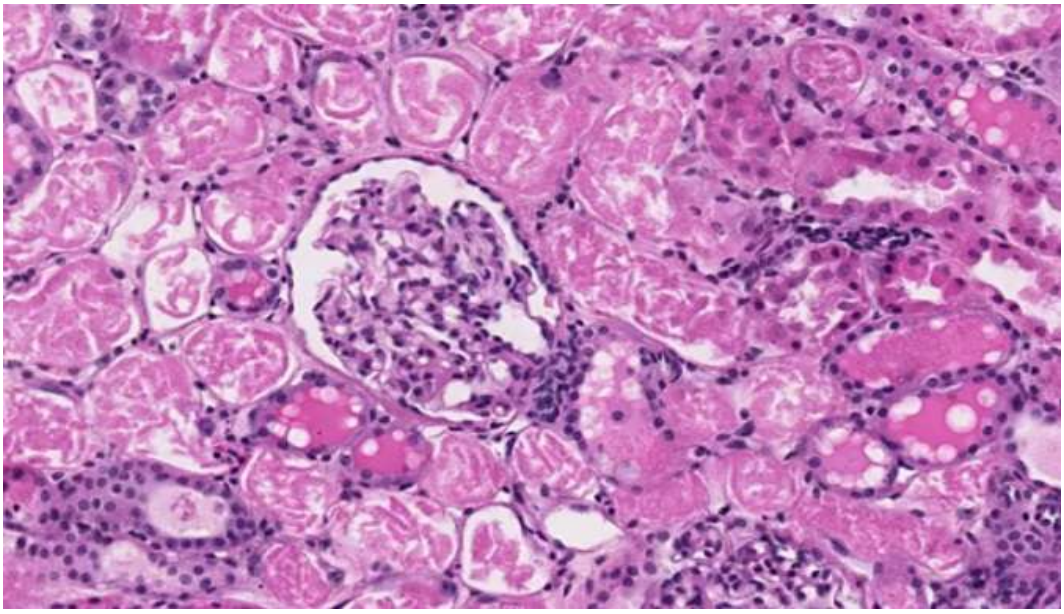

Figure 5 Kidney section from rat treated with gentamicin showing necrosis and degeneration of glomerulus**.**

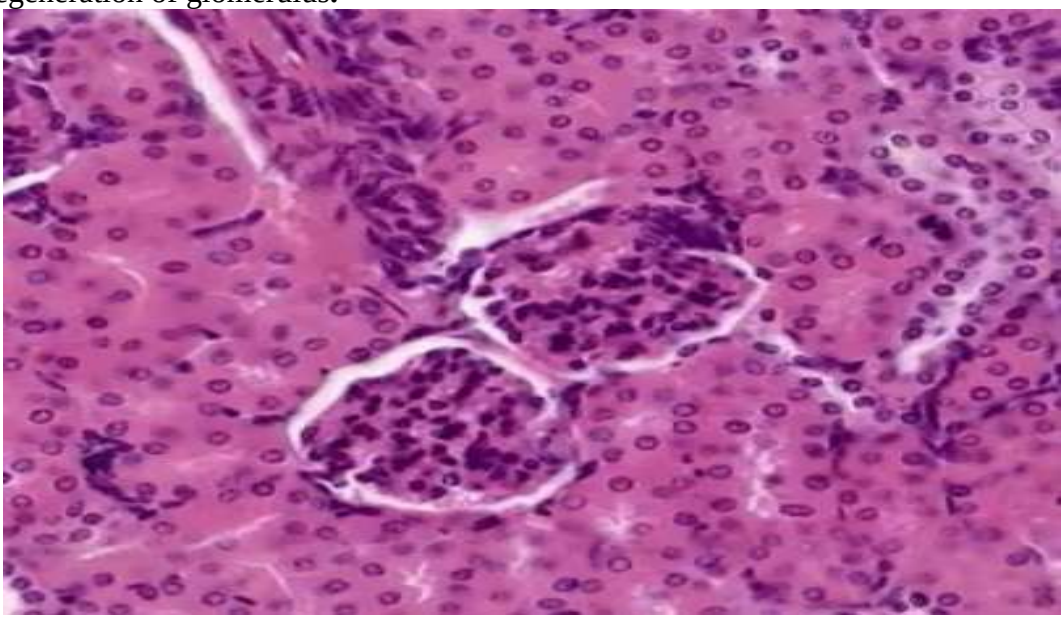

Figure 6 Kidney section from rat treated with *Terfezia claveryi* extract showing normal morphology when compared with control rat.

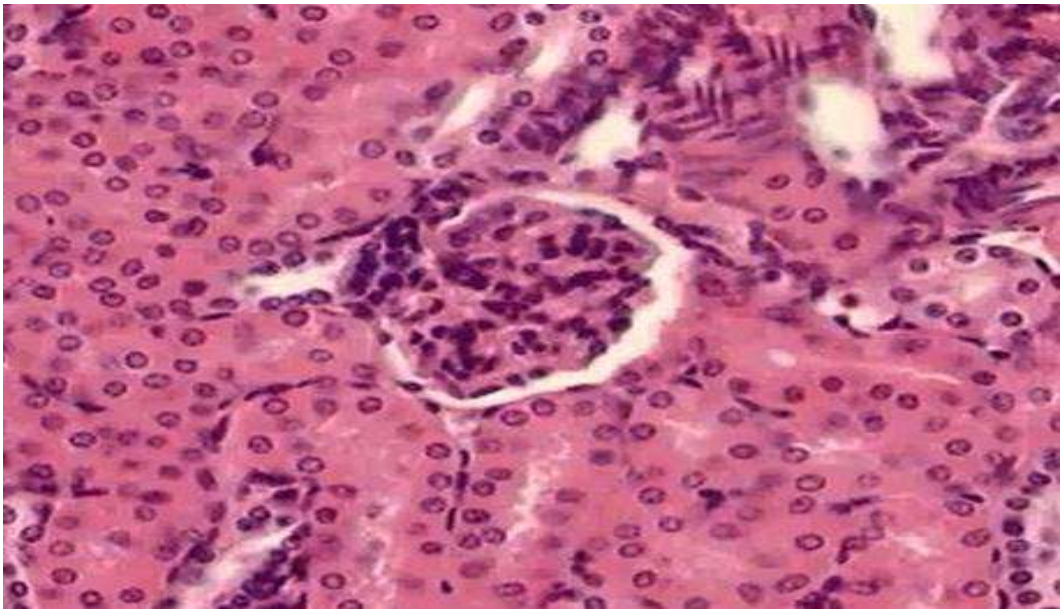

Figure 7 Kidney section from rat treated with gentamicin and *Terfezia claveryi* extract showing a reduction of necrosis and degeneration of glomerulus.

Discussions:

In this study the administration of gentamicin significantly increase the serum urea and creatinine concentration, and this indicate kidney dysfunction (29) and this result are in agreement with several studies(30, 31).

The increase in serum biochemical parameters were well directly correlated with the renal histological damage.

There have been many studies in recent years suggesting a significant role for reactive oxygen species (ROS) in gentamicin induced nephrotoxicity (7). Sha & Schacht (32) have suggested that aminoglycoside antibiotics can stimulate formation of free radicals in renal cortical mitochondria and glomerula . In addition, ROS scavengers and antioxidants are used to ameliorate the gentamicin induced nephrotoxicity (33, 34). Superoxide dismutase treatment has shown some promise in protection against gentamicin induced nephrotoxicity in rats (35).

The major causative factor of tissue damage is lipid peroxidation which is motivated by the ROS formation, and this process is associated with the formation of MDA because it is the by-product of lipid peroxidation (36-38).

Our results show the increase in the MDA formation in significant trend when we compare it with the control group and this indicate the administration of gentamicin induce free radicals generation and this radicals initiate the lipid peroxidation process, previous studies also reported the direct relationship between the oxidative stress and MDA formation(39-41).

The ROS induced by gentamicin and the free radicals of the propagation step of the lipid peroxidation will propagate the oxidative stress in the rat and this will lead to the depletion of the antioxidant enzymes to neutralize the oxidative stress of reactive oxygen species and this is compatible with our results and other results when they studies the correlation between the oxidative stress and the antioxidant enzymes, therefore our results show decreasing in the activity of the antioxidant enzymes(42-47).

The administration of the *Terfezia claveryi* extract to the group that resaved gentamicin lead to reduce the oxidative stress due to the antioxidant properties of the *Terfezia claveryi* (16, 17). The protective effects are done by several mechanisms like

scavenger of free radicals, lipid peroxidation chain breaker, recycling of non-enzymatic antioxidant molecule, and chelating the redox active metal that enhance the formation of ROS by Fenton reaction (48-60)

The *Terfezia claveryi* properties cause a reduction of the aggressiveness renal defect from gentamicin via reducing the formation of the ROS that cause lipid peroxidation (this mean the reduction of the MDA) lead to maintain the integrity and fluidity of the cell membrane. And our result shows the reduction of MDA formation after co-administration with *Terfezia claveryi* and reduction of formation of ROS. On the other hand co-administration of *Terfezia claveryi* decrease the oxidative stress load, therefore the antioxidant enzymes relatively returned to best value compared with the enzymes activity of gentamicin alone.

Conclusion

The administration *Terfezia claveryi* extract on the experimental animals had an oxidative stress induced by gentamicin will reduce the production of MDA which is positively correlate with lipid peroxidation on the other hand *Terfezia claveryi* cause an increase in the activity of SOD, CAT, and GPX on kidney homogenate and ameliorate the histopathological defects that caused by gentamicin.

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