Effect of *Olea Europea (* Olive oil) on gentamicin induced hepatorenal toxicity in male rats

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Abstract

Gentamicin is aminoglycoside antibiotic commonly used for the treatment of Gram-negative bacterial infection. In many cases, it has been the only effective therapeutic drug against bacterial strains resistant to other antibiotics, but nephrotoxicity and hepatotoxicity side effects limit its use. The aim of this study is to investigate the protective effect of olive oil against gentamicin-induced hepatorenal toxicity in male albino rats. In this study we used 24 wistar-albino rats and divided them into 4 equal groups. Each experimental group consisted of six animals. group1,control they were given normal saline only ,group2,gentamicin 100 mg/kg/day intraperitoneal (IP),group3,olive oil 5 ml/kg/day (oral administation) ,group 4,gentamicin 100 mg/kg/day intraperitoneal (IP) and olive oil 5 ml/kg/day (oral administration).Treatments were administered once daily for 21 days. After 21 days, biochemical and histopathological analysis were conducted to evaluate hepatoranal toxicity. Serum levels of urea, creatinine, cholesterol,triglyceride,and activity of AST and ALT were measured.Animals treated with gentamicin alone showed a significant increase in serum levels of these markers.Treatment of rats with olive oil showed significant improvement in renal and hepatic function , presumably as a result of decreased boichemical parameters associated with gentamicin-induced hepatorenal toxicity.Histopathological examination of the rats kidneys and liver confirmed these observations. Therefore olive oil may protect against gentamicin-induced hepatorenal toxicity

أثر زيت الزيتون على التسمم الكلوي الكبدي المستحث باعطاء الجنتاميسين لذكور الجرذان

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الخلاصة:

مفتاح الكلمة:الجنتاميسين,زيت الزيتون,السموم الكلوية الكبدية,الجرذان

الجنتاميسين هومضاد حيوي (امينوكلايكوسايد) يستخدم عادة لعلاج الاصابات البكتيريةالسالبة لصبغة غرام. في كثير من الحالات، اذ كان الدواء العلاجي الفعال الوحيد ضد سلالات بكتيرية مقاومة للمضادات الحيوية الأخرى، ولكن التسمم الكلوي الكبدي تحد من استخدامه. الهدف من هذه الدراسة هو التحقيق من الآثار الوقائية لزيت الزيتون ضد التسمم الكبدي الكلوي التي يسببها الجنتاميسين في الفئران عن طريق قياس بعض المعايير الكيميائية الحيوية والتشريح النسيجي لأنسجة الكلية والكبد. تضمنت الدراسة استخدام 24من جرذان الوستر البيضاء - وقسمناها إلى اربعة مجاميع متساوية على النحو التالي: المجموعة الاولى، السيطرة؛ المجموعة الثانية، جنتاميسين 100 ملغ / كغ / يوم، داخل الصفاق(البريتون) ، المجموعة الثالثة زيت الزيتون 5ملم/ كغ / يوم، (عن طريق الفم)؛ المجموعة الرابعة، جنتاميسين 100 ملغ / كغ / يوم، داخل الصفاق وزيت الزيتون 5ملم/ كغ / يوم (عن طريق الفم). وكانت العلاجات تعطى مرة واحدة يوميا لمدة 21يوما. و اجريت التحاليل الكيميائية الحيوية والنسيجية المرضية بعد 21يوم لتقيم التسمم الكبدي الكلوي . أثبتت نتائج التجربة بأن اعطاء مادة الجنتاميسين للجرذان ولمدة 21يوما سببت زيادة ملحوضة في مستوى الدهون في الدم و زيادة مستوى فعالية انزيمات وظائف الكبد و ارتفاع مستوى اليوريا والكرياتينين بينما لوحظ ان اعطاء زيت الزيتون للجرذان المحقونة بدواء الجنتاميسين أدت الى انخفاض في القياسات الكيميائية الحيوية اعلاه. وقد تم التوصل بأن هناك تغيرات نسيجية مرضية في أنسجة الكبد والكلية في الجرذان المحقونة بمادة الجنتاميسين في حين قلت التغيرات النسيجية المرضية بعد اعطائها زيت الزيتون.

Introduction:

Gentamicin is a widely used aminoglycoside antibiotic with a low price and high effectivity in treatment of gram-negative and β-lactam resistant infections [1]. However, its use has been limited because of its adverse effect of causing nephrotoxicity [2]. Gentamicin-induced nephrotoxicity is an animal model for study of acute kidney failure in experimental research [3]. Although the mechanism by which gentamicin causes nephrotoxicity remains unclear, proposed pathological mechanisms include induction of oxidative stress, apoptosis, necrosis, elevation of endothelin I and increase of monocyte/macrophages infiltration [3–5]. Gentamicin-induced nephrotoxicity is clinically characterized by increased serum creatinine and blood urea nitrogen and decreased glomerular filtration rate [2,3] ,and morphologically by focal necrosis and apoptosis in tubular epithelium with extensive peritubular cell inflammation [2]. Gentamicin increases generation of reactive oxygen species (ROS), such as super oxide anions [6], hydroxyl radicals, hydrogen peroxide and reactive nitrogen species in the kidney [3].

The olive tree, *Olea europaea*, produces the olive fruit. Olives are grown widely in the Mediterranean basin and parts of Asia Minor. Historically, the products of *Olea europaea* have been used as aphrodisiacs, emollients,

laxatives, nutritives, sedatives, and tonics. Specific conditions traditionally treated include colic, alopecia, paralysis, rheumatic pain, sciatica, and hypertension[7]. Olive oil is believed to exert its biological benefits mainly via constituent antioxidants. Although the composition of olive oil is complex, the major groups of compounds thought to contribute to its observed health benefits include oleic acid, phenolics, and squalene,[8] all of which have been found to inhibit oxidative stress. Antioxidants in olives protect them from oxidation by the high temperatures and ultraviolet radiation of the Mediterranean climate [9].

The aim of this study is to investigate the protective effects of olive oil against gentamicin -induced hepatorenal toxicity in rats by biochemical assaying and histopathology of kidney and liver tissues.

Material and Methods:

Chemicals:

Gentamicin (80 mg/2ml) was obtained from the Essential Drug Company (Baghdad, Iraq),and given as intraperitoneally (IP)at a dose of 100mg/kg body weight as previously described [10] . Olive oil was purchased from local market (Karbala, Iraq). Olive oil was given by gavages at a dose of 5 ml/kg as described [11] .

Animals:

In this study, we used 24 Wister albino 210-230 g male rats which were housed in wire bottom cages, free diet, tap water and with a 12 h light/ dark cycle for 3 weeks. The experimental protocol and procedures used in this study were approved by the Ethics Committee of the Kerbala University, Kerbala, Iraq for the care and use of laboratory animals. The animals were randomly divided into 4 groups. Each experimental group consisted of six animals.

Group 1, Control group (n= 6): They were given only normal saline for 3 week.

Group 2, Gentamicin (G) (n= 6): Animals of this group were given gentamicin as intraperitoneal (IP) at a dose level of 100 mg/kg body weight, every day for 3 weeks.

Group 3, Olive Oil (OO) (n= 6): Animals of this group were given olive oil via gavage at a dose level of 5mL/kg body weight, every day for 3 weeks.

Group 4, Gentamicin + Olive oil-treated group: Rats were treated with gentamicin (IP) (100 mg/kg) and Olive oil (oral administration) (5mL /kg) daily for 3 weeks.

The end of the experiment rats were given cloroform for anesthesia and were sacrificed 24h after the last olive oil and gentamicin received, and blood samples were collected in centrifuge tubes. Serum was separated from coagulant blood by centrifugation at 860 g for 20 min, and then frozen at -8°C for biochemical analysis. The kidneys and liver were excised and the specimens were fixed in formalin 10 % solution for 72 hours. After fixation, the tissues were washed under running tap water for 24 h and dehydrated with 50, 60, 70, 80, 90, 96 and 99% concentrated ethanol. The specimens were then laid in a 1:1 ratio of immersion oil and absolute alcohol for 1h, followed by immersion oil overnight, for transparency. After the application of xylol, the specimens were made into paraffin blocks using a 1:1 xylol and paraffin mixture for 1h and paraffin for 6h in an incubator. 10 micron thick sections were rehydrated and dyed with Masson's trichrome technique [12].

Biochemical analysis:

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were estimated according to Reitman and Frankel methods.[13]Creatinine levels was determined using Diamond diagnostic kit according to Owens et al.[14]Where as urea was estimated by the use diamond daignostic kit as previously reported.[15] The cholesterol and triglycerides were estimated by the use biolabo daignostic kit as described .[16,17]

Statistical analysis:

The data was analyzed using the Statistical Package for Social Science program (SPSS 12). For comparison between different experimental rat groups, one way analysis of variance (ANOVA) was used followed by Tukey's test. The results were expressed as means ± MSE and $P < 0.05$ was considered to be statistically significant.

Results:

As shown in table 1. Gentamicin increased the level of liver biochemical parameters; AST, ALT in serum as compared to that of the control group . Treatment of olive oil in combination with gentamicin reduced the serum level of AST, ALT to that of the gentamicin treated group. Treatment of oilve oil (5 ml/kg bw) alone did not induce significant ($P>0.05$) change in the level of liver marker enzymes as compared to control group. Gentamicin also increased the level of kidney biochemical markers; urea, creatinine in serum as compared to thats of control group. Co-adminstration of olive oil and gentamicin reduced the serum level of urea and creatinine to significant ($p>0.05$). The level of cholesterol and triglyceride significantly increased in group that are treated with gentamicin while the level decreased significantly ($p>0.05$) in group that are treated

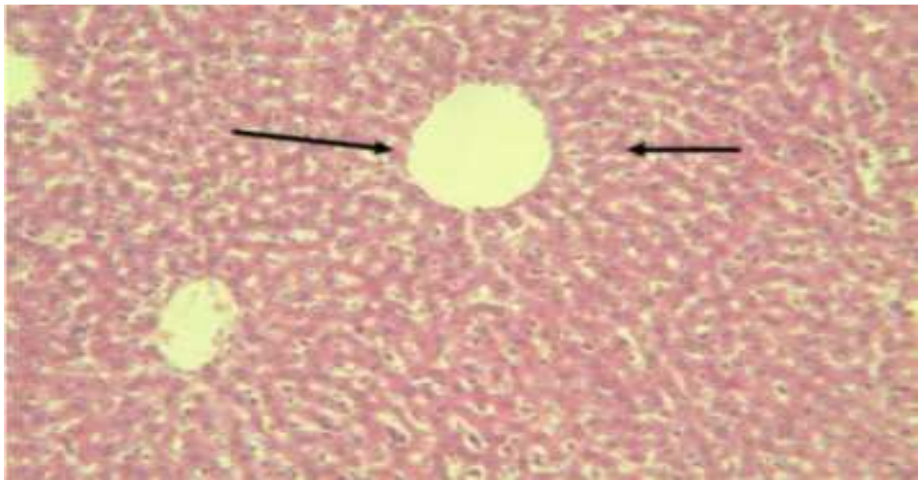
with gentamicin and olive oil and no significant increase in level in group treated with olive oil alone.

Table 1: The levels of serum AST , ALT, Urea, Greatinin, cholesterol and triglyceride in control group , gentamicin treated group , olive oil treated group, gentamicin and olive oil treated group rats.

Groups	AST (U/ml)	ALT (U/ml)	Urea(mg/dl)	Creatinine (mg/dl)	Cholesterol (mg/dl)	Triglycerides (mg/dl)
Control	**74.96±7.17**	**20.6± 3.13**	**17.67±3.24**	**0.66±0.17**	**7.23±0.55**	**12.66±1.92**
Gentamicin	**222.36±23.53***	**81.78±6.79***	**51.62±7.03***	**1.71±0.24***	**20.4±1.94***	**24.25±2.65***
Olive oil	**74.83±8.52**	**20.28±2.97**	**17.29±4.12**	**0.65±0.15**	**6.71±0.67**	**10.6±1.55**
Gentamicin+ Olive oil	**75.3±9.59**	**21.66±5.44**	**18.60±2.79**	**0.67±0.19**	**7.83±0.73**	**13.75±1.47**

Values are expressed as mean ± SE
***Level of significance p < 0.05.**

The histology of the liver and kidney from control and olive oil-treated animals showed normal histological morphology(figures 3,4,7,8),whereas in animals treated with gentamicin showed tissue destruction along with hyperemia and congestion of centrilobular vein and congestion, severe degeneration of tubular cells of kidneys. (figures 5,9).this effect was relatively decreased in animals co-administared with olive oil(figures 6,10).



Figure(3) Optical photomicrographic of liver tissue (H & E staining, magnification × 400).The figure shows photomicrograph of live tissue in group (1) that received only saline . Normal liver tissue without any sign of necrosis (control group). Hepatocytes (right arrow), centrilobular vein (left arrow).

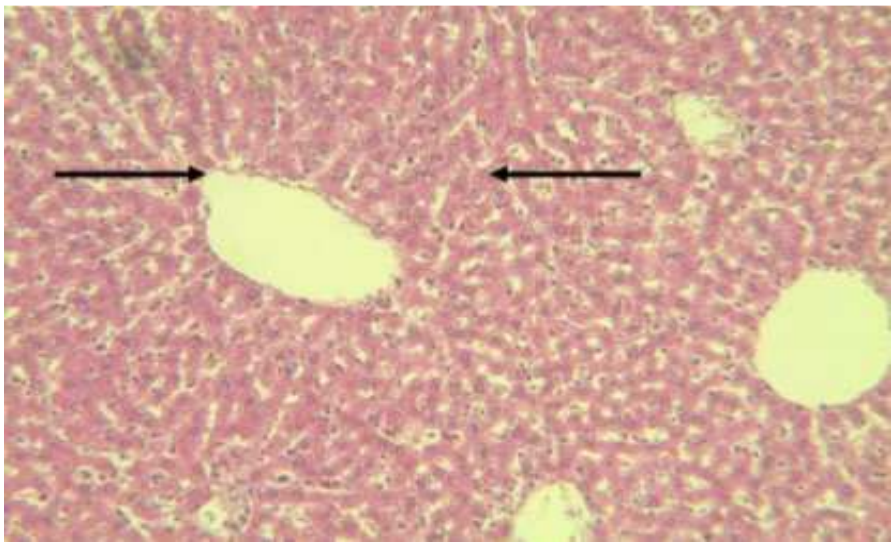


Figure (4) Optical photomirograph liver tissue (H & E staining, magnification × 400) The Figure below shows liver tissue in group(2) that received the olive oil . Normal liver tissue without any sign of necrosis Hepatocytes (right arrow), centrilobular vein (left arrow) .

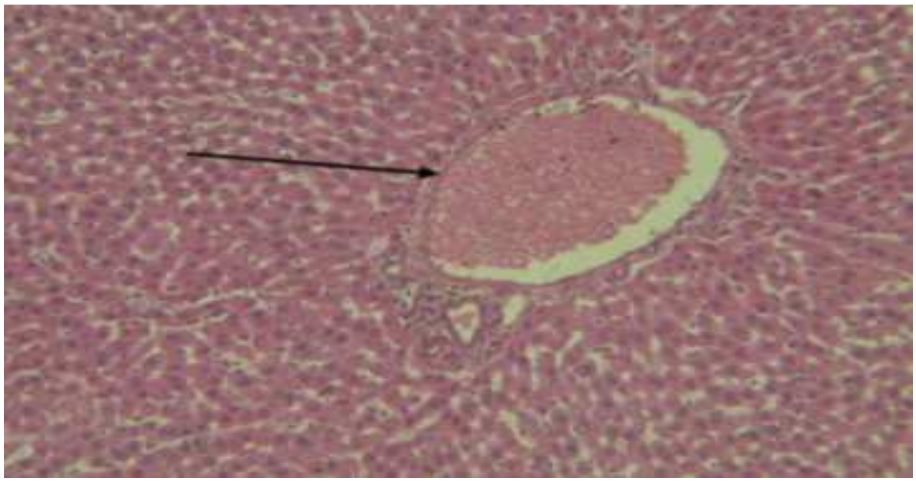


Figure (5) Optical photomicrograph of liver tissue (H & E staining, magnification × 400) liver tissue damage in group (3) receiving Gentamicin as 100mg/kg The Figure below shows photomicrograph of liver tissue (G) that used gentamicin as 100mg/kg BW. tissue destruction along with hyperemia and congestion of centrilobular vein.

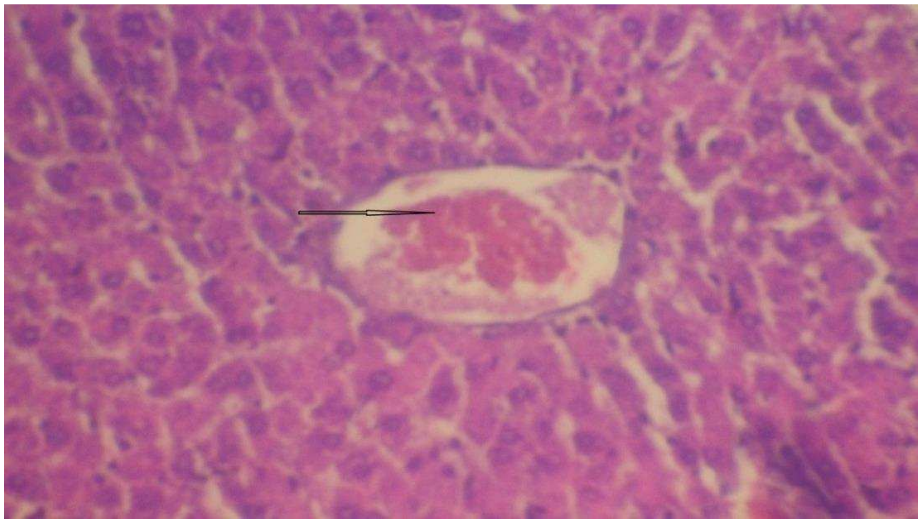


Figure (6) Optical phoromicrograph of liver tissue (H & E staining, magnification × 400) liver tissue in group that received gentamicin and olive oil. Figure shows photomicroghraph of liver tissue in group (3) that received gentamicin and olive oil , Decrease of congestion region.

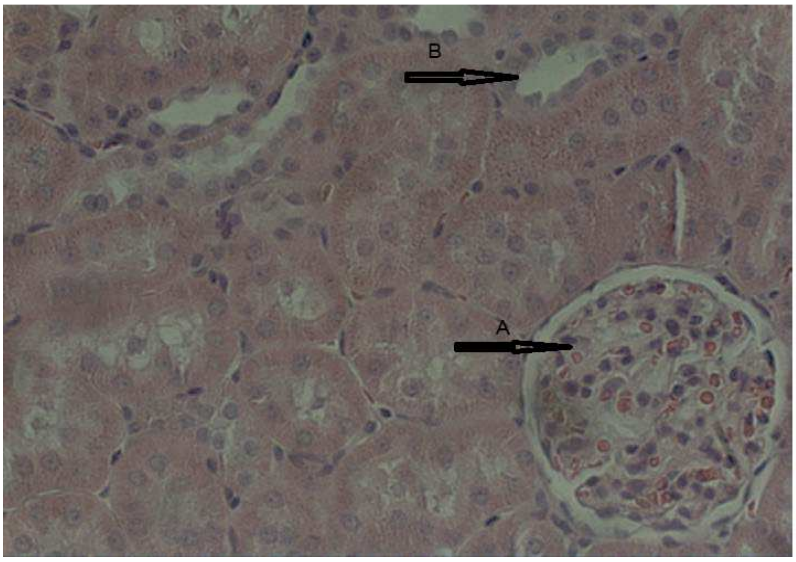


Figure (7) Kidney section fromcontrol group (1)(H&S 200X) Shows (A) normal histological structure of the glomeruli and (B) renal tubules.

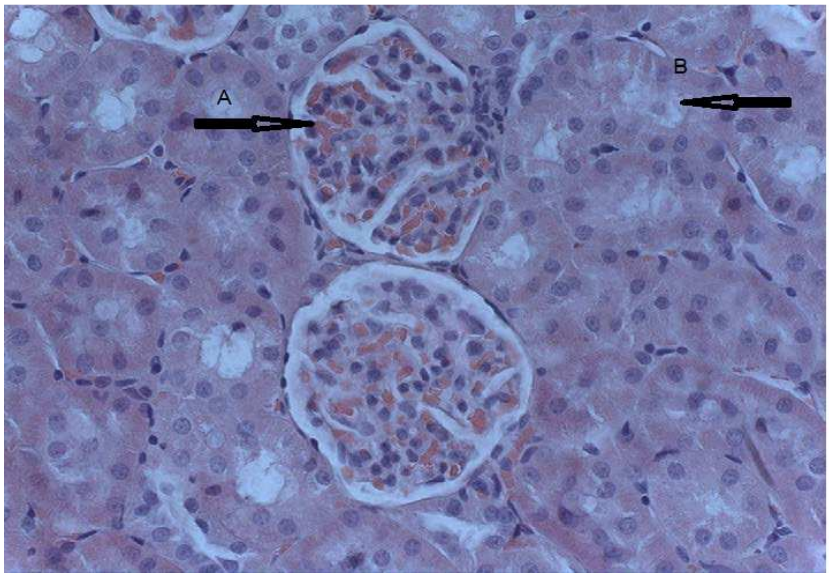


Figure (8) Kidney section from group (2)(H&S 200X) that was treated with olive oil showing normal morphology when compared with control rats ,(A) normal glomerulus and (B) renal tubules.

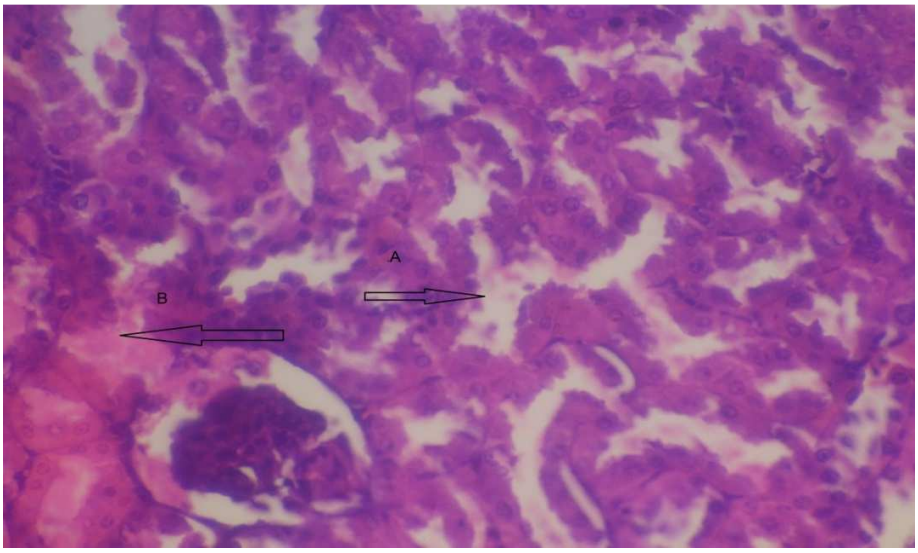


Figure (9) Kidney section from rat treated with gentamicin group (3)(H&S 200X) showing(A) severe degeneration of tubular cells and(B) severe congestion .

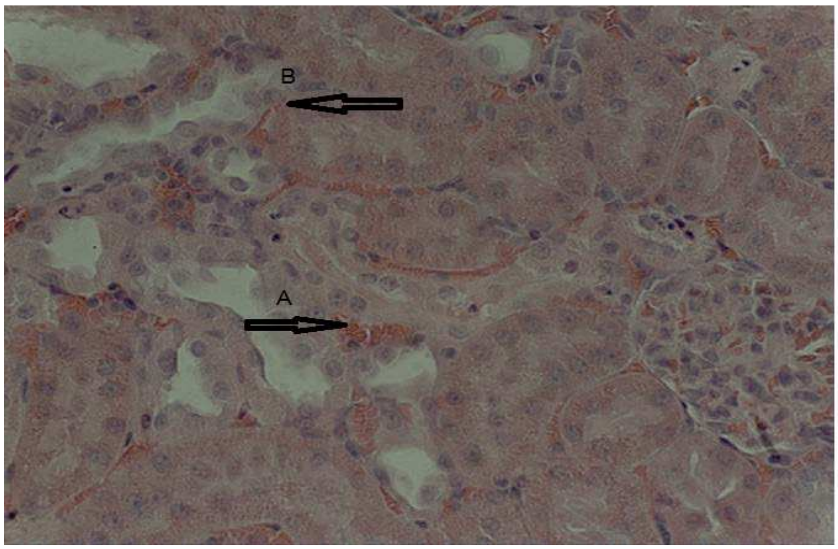


Figure (10) Kidney section from rat treated with gentamicin and olive oil group(4)(H&S 200X) showing a A)reduction in congestion and (B)tubular degeneration.

Discussion

Gentamicin is a therapeutic agent used against the life threatening infections in human, but it causes acute renal failure in 10-15% of the patients [18]

while more than 30% of the patients showed the signs of nephrotoxicity which have received the gentamicin for more than 7 days [19,20]. Also important complication gentamicin includes liver toxicity (hepatotexicity) [(21,22)]. In this study we have tested the hypothesis that olive oil administration could prevent gentamicin induced hepatorenal toxicity in rat. Hepatocytes are complex metabolical liver cells that contain large amounts of enzymes. These enzymes are poured into the plasma due to liver damage, and can be useful for the detection and determination of liver damage. A recent report suggested GEN generates ROS which could induce apoptosis in rat liver cells and finally leads to liver failure [23] .GEN-induce an increased lipid peroxidation , which serves as substrate for free radical attack [24]. Lipid peroxidation is an oxidative stress which takes place in the cell membranes or tissues resulting in increased production of ROS/decreased antioxidants which leads to an imbalance between oxidant and antioxidant status and ultimately leading to cellular damage. Gentamicin treatment causes significant increase in the serum activity of liver function markers as compared to control group, indicating hepatic dysfunction. These dysfunction may be due to the liver damage that is one of the major factors of liver inefficiency in a significant number of people taking this medication. Therefore taking these medications face limitations due to the fact that one of the major side effects of Gentamicin is creating hepatotoxicity [25-27]. Increased production of Reactive Oxygen Species (ROS), which can be seen after the use of gentamicin in cells, is effective in inducing toxic impacts of this drug on the structure and function of tissues. The results from this study confirmed that gentamicin at a dose of 100 mg/kg/day for 3 weeks produces significant hepatotoxicity as evidenced by increase in serum AST and ALT. Transaminases (AST and ALT) were considered to be a more sensitive measure in evaluating liver function and damage [28]. Hatoff [29] reported that elevations in serum levels of these enzymes were mostly attributed to acute hepatocellular damage or extrahepatic obstruction, or both. These enzymes were secreted to blood in hepatocellular injury and their levels increased. This results obtained in this study are agree with other reports showing elevations of these enzymes in experimental animals exposed to Gentamicin[30]. Gentamicin exposure to rats mediates the generation of ROS that play a significant role in the progression of hepatic and renal injuries including array of biomolecules such as membrane lipids, protein and nucleic acids especially in some organelles such as mitochondria and lysosomes of renal tissues [24]. Increased propagation of ROS mediates the peroxidation of polyunsaturated fatty acids, attached to biomembranes. Gentamicin induced lipid peroxidation impaired the cellular function and cause necrosis. In this study we observed a significant increase in the lipid peroxidation [24].

Gentamicin treatment is found to elevate creatinine and urea levels in serum. These observations are generally in agreement with other studies [31,32]. Creatinine and urea are waste products of protein metabolism that need to be excreted by the kidney, therefore a marked increase of these parameters, as observed in this study, confirms an indication of functional damage to the

kidney[33]. Urea level can be increased by many other factors such as dehydration, antidiuretic drugs and diet, while creatinine is more specific to the kidney, since kidney is the only significant factor that increases the serum creatinine level [34]. The increase in creatinine recorded in this work might be due to impaired kidney function by the used antibiotic. This view was supported by Kluwe, [35] who indicated that an elevation of creatinine level in the blood is an indicative of impaired kidney function. It can be speculated that amelioration of creatinine clearance by olive oil is due to the recovery of tubular and glomerular function in gentamicin nephrotoxicity. In the present study, the gentamicin-treated rats showed severe proximal tubule necrosis. Tubular necrosis was inhibited in the animals treated with gentamicin and olive oil in comparison with gentamicin-only treated animals. Such results were reported by other researchers by using antioxidant compounds previously [36-40].

Lipid profile changes are associated with the phenomenon that excessive load of fat fractions (cholesterol to the liver above the acceptable level of its normal physiological limit, causes the liver to be unable in metabolizing the lipids, therefore resulting in high cholesterol return to the blood circulation [41]. Studies in both animals and humans have demonstrated that prolonged high cholesterol concentration in the circulating blood positively correlates with developing atherosclerosis [42, 43]. In this study , however the treatment of hyperlipidemic rats with Olea europea recorded a remarkable reduction of lipid profile , but also a protective effect against atherosclerosis as indicated by a reduced AI value . So, the present data demonstrated that the supplementation of studied oil for hyperlipidemic rats were inhibited the elevation of serum lipid fractions. The lipid lowering effects of the studied oil possesses lipid lowering properties. The mechanism on how exactly the oils could lower blood lipid fractions requires further investigation, but it was postulated that high polyphenolic , flavonoids and sulfhydryl compounds concentrated in the studied oil preparations could partly explain the underlying mechanism of its lowering properties. The mechanism of serum lipid lowering belonged to delayed lipid absorption from GIT and diminished LDL-C synthesis by the liver [44].

Conclusion:

In the present study, AST, ALT activity, urea, creatinine, cholesrerol and triglyceride levels were analyzed, it was concluded that olive oil has significant hepatorenal protective activity against gentamicin- induced hepatorenal toxicity in rat.

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