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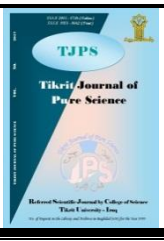

Protective effect of citrus honey toward the histopathological and biochemical nephrotoxicity damages of gentamicin–induced in local rabbits *Oryctolagus cuniculus*

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ABSTRACT

Drugs induced nephrotoxicity now considered as an essential reason for kidney failure. Aminoglycoside anti-infection agents, for example, gentamicin, which causes ototoxicity and nephrtotoxicity as a side effect , this research is centered around the utilization of natural materials as an antioxidants against the lethal oxidative activity that applied on the kidney cells ,the most imperative one of these materials is the honey. This work aimed to assess the defensive impact of citrus honey against the histopathological and biochemical harms of gentamicin induced nephrotoxicity .24 locally breed rabbits (***Oryctolagus cuniculus***) were used and divided into 4 study groups (6 rabbits per each group), group 1 received I.P daily dose of normal saline (control), group 2 received (80 mg/kg/day) I.P dose of gentamicin, group 3 received (80mg/kg/day) of gentamicin I.P dose in combination with oral dose of Citrus Honey625 mg/kg/day for 14 days and group 4 received (80mg/kg/day) of gentamicin I.P dose in combination with extra dose of Citrus Honey orally for 14 days. All animals (at 15th day) were anesthetized by ether and sacrificed; blood samples were gathered for the subsequent measurement of the serum creatinine, urea and albumin while an isolated kidney was kept in 10 % of formaldehyde for the histopathological examination. The results demonstrated that gentamicin causes nephrotoxicity showen by elevation of serum level of creatinine, urea and a decrease in the serum albumin. While the administration of honey in combination with gentamicin reduced the nephro-toxic effect of gentamicin that represented by a reduction of the serum creatinine and urea with improvement of the kidney histological findings.This study concluded that, honey decreased nephrotoxic effect of gentamicin.

Introduction

The utilization of nephrotoxic anti-infection agents has been ensnared as a causative factor in 25% of all causatives of kidney failure in critically ill patients[1]. This is likely in light of the fact that the kidney provided with a large volume of blood representing 20% of total cardiac output, as a result the kidney is probably going to be influenced by antibiotics and their metabolites that are accumulated through the urine concentrating mechanism[2]. Aminoglycosides are powerful bactericidal anti-microbials; they act especially against aerobic, gram-negative bacteria.[3] Gentamicin is one of the aminoglycoside, generally used for treatment of serious, emergency clinic obtained diseases with multidrug resistant gram negative bacteria such as *Pseudomonas aeruginosa*, *Acinetobacter*, and *Enterobacter*[4]. Aminoglycoside induced nephro and ototoxicity,which are the limiting factors for their clinical [5]. Wojckch Lesniak, *et.al.*[6]found that aminoglycosides, exert their adverse renal effect by generation of reactive oxygen species. Additionally, it has been shown that aminoglycoside form a complex with mitochondrial Fe^{+2} to form a free radicals.[7]

Honey is sweet, thick syrup made by honey bees from nectar of flowers. The plants flowers from which bees gather nectar is the main thing that determine the color, flavor, and smell of honey[8].

Honey considered a highly saturated water solution of sugar, which includes a mixture of highly complexed carbohydrates, enzymes, amino acids, organic acids, minerals, aromatic substances, pigments, wax and pollen.[9] .Studies reported that honey also possesses natural antioxidants through many compounds like vitamin C and polyphenols like chrysin, pinobanksin, luteolin and pinocembrin that can decrease oxidative stress in humans[5, 9,10].

The aim of this study is to evaluates the possible protective effects of honey against nephrotoxicity induced by gentamicin.

Materials and methods

24 Adult male local rabbits ***Oryctolagus cuniculus*** weighting between 1000gm and1700gm were used under states of controlled temperature and humidity. The animals were fed commercial pellets and tap water. The honey used in this study was the *citrus* honey .It was given to animals by oral gavages tube in a dose of 625mg/kg/day.[9]

Experimental design

Group1- Six rabbits were treated with I.P injection of normal saline for 14 days .This group served as control.

Group 2– Six rabbits were treated with I.P injection of 80 mg/Kg/day of gentamicin for 14 days .This group served as positive control for nephrotoxicity induced by gentamicin .

Group 3- Six rabbits treated with oral dose of 500mg/kg/ day of honey concomitantly with I.P dose of gentamicin (35mg/kg/ day) for 14 days.

Group 4 received (80mg/kg/day) of gentamicin I.P dose in combination with extra dose of Citrus Honey orally for 14 days.

Group 3 and 4 used to investigate the possible protective effect of honey against nephrotoxicity induced by gentamicin . All animals were anesthetized by ether and sacrificed after 2 weeks of treatment.

Preparation of blood samples and tissue

After 2 weeks of treatment, the blood samples were collected before the animals had been sacrificed. Blood samples were clotted then centrifuged at 3000 rpm. for 15 minutes .Serum was stored at -20ºC until used for the determination of creatinine [11], urea [12]and albumin[7], while the kidney was kept in formaldehyde(10%) and used for histological examination using paraffin section technique.[13] .Statistical analysis performed using unpaired Student´s t-test. Data were presented as mean± SD, P-values less than 0.05 were considered significant for all the results in this study.

Results

Effects on the histology of the kidney

Group 1: The kidney of adult male rabbits appeared fully developed, with a thick cortex. The glomeruli formed by several tufts of capillaries. Cuboidal epithelium are lining the proximal convoluted tubules with normal borders. Normal renal glomeruli are surrounded well-developed capsules. While the distal convoluted tubules. The cortical collecting tubules have larger lumen, and in their transvers sections their profiles are circular in contrast to the irregular shape of the convoluted tubules .While the cortex collecting tubules have larger caliber as shown in Figure 1.

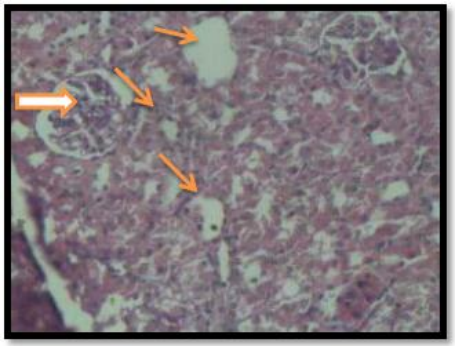

Fig. 1: Kidney of control group showed glomerulus (thick arrow) & convolute tubules (thin arrows) (H&E. 400X).

Group 2: Gentamicin – treatment of adult rabbits causes vacuolation, hydrophic degeneration, desquamation and necrosis in epithelial cells of the convoluted tubules. Hyaline casts are obvious in the convoluted tubules lumen. Haemorrhage was clear. Changes in the glomerulus such as congestion,as shown in Figure 2.

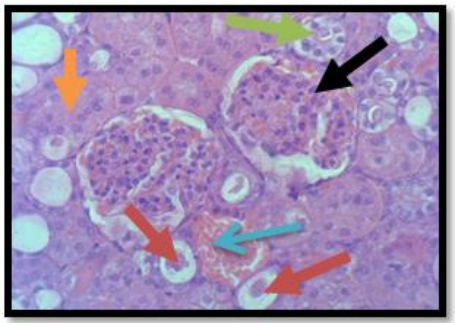

Fig. 2: Rabbit kidney cortex treated with Gentamicin (GM) hypercellularity (blacke arrow), hydrophic degeneration(orange arrow), haemorrhage (blue arrow) and Hyalinization(red arrows) desquamation (green arrows) (H&E.400X).

Groups 3 and 4: All Gentamicin and honey treated groups revealed almost normal glomeruli with intact basement membranes of their Bowman's capsule. Some glomeruli were slightly deformed. The convoluted tubules showed intact basement membranes. While, there was little swelling of the lining epithelium of some convoluted tubules with the presence of some hyaline casts. Also a slight wideness of the urinary space was observed. Scattered mononuclear inflammatory infiltration was seen, as shown in figures 3 and 4.

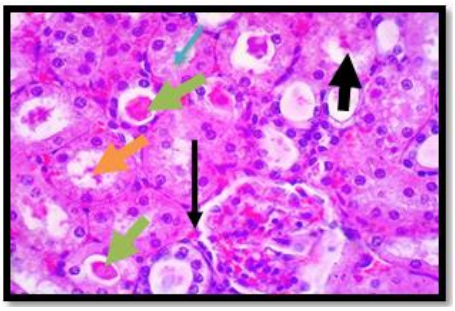

Fig. 3: Rabbit kidney cortex treated with (C.H 625mg/dl & GM) for 15 days, degeneration (thick black arrow), congestion (thin black arrow), necrosis (blue arrow), hyalinization (green arrows) and proteinaceous materials (orange arrow). (H&E.400X).

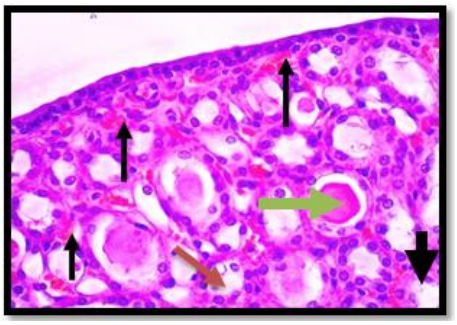

Fig. 4 : Renal cortex of Rabbit treated with GM & C.H extra. for 15 days, shows congestion (thin balck arrows), hyalinization (green arrow),necrosis (thick black arrow), and proteinaceous materials (red arrow).(H&E.400X).

Renal function tests and Albumin

The results of this study showed significant increase ($p<0.05$) in the serum levels of both creatinine and urea while there was significant decrease in albumin of animals treated with 80 mg/kg/ day of gentamicin (group 2) compared to the corresponding levels in the control animals (group 1) .

There were significant decrease ($p<0.05$) in the serum levels of both creatinine and urea and significant increase in albumin of Rabbits treated with 80 mg/kg/ day of gentamicin + 625 mg/kg/day of honey (group 3) compared to the corresponding levels of Rabbits treated with 80 mg/kg/ day of gentamicin (group 2). (Table 1, figure 5 , figure 6 and figure 7).

Table 1 Effect of gentamicin on the serum urea , creatinine and albumin (n=6)

Groups	Creatinine *mg/dl*	Urea *mg/dl*	Albumin *mg/dl*
Group 1 X ± SD	0.73 ±0.06	24±4.13	3.64±0.05
Group 2 X ± SD	0.93 ± 0.10 *	45.8± 4*	2.13 ± 0.04*
Group 3 X ± SD	0.5 ±0.02*	37±3.5*	3.35±0.06
Group 4 X ± SD	0.72±0.028	38.75±3.7*	4.4±0.21 *

X= mean, SD= standard deviation, n=number of animals, * =significant ($p<0.05$)

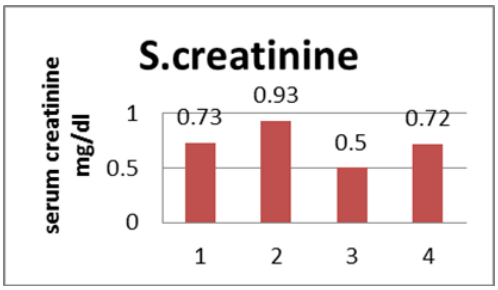

Fig. 1: Serum creatinine levels of experiment animals groups .

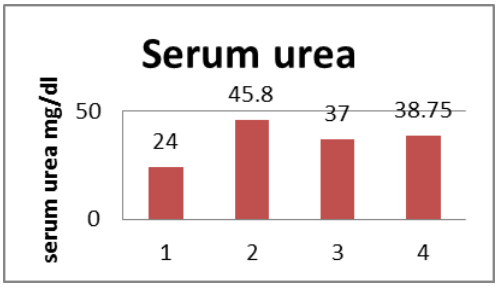

Fig. 2: Serum urea levels of experiment animals groups

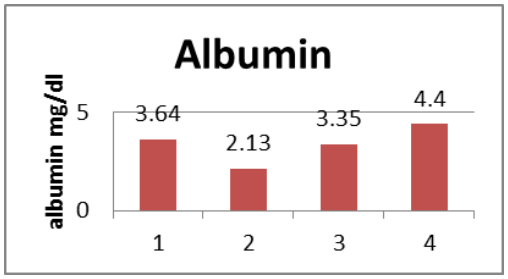

Fig. 3: Albumin levels of experiment animals groups

Discussion

The administration of gentamicin caused nephrotoxicity, which was characterized by the significant elevation of serum creatinine and serum urea. This was accompanied by the tubulonephritis when compared with the control group. Gentamicin grouped in the kidney cortex due to its reabsorption in the proximal]tubules causing degeneration and necrosis of the tubules epithelial cells, similar to what was observed in the present study [14].

The pathway by which tubular damage was caused by gentamicin was reported to be due to oxidative injury and lipid peroxidation [15]. Baliga *et al.* reported that, the entrance of gentamicin to the renal proximal tubules was due to interaction between anionic cell membrane phospholipids and the cationic drug.Then this initiates the cortical mitochondria to release iron, which forms iron – drug complex, which leads to the formation of free radicals. Reactive oxygen species (Ros) attacks DNA which cause eventually a damage in the kidney through changing the contraction of mesangial cells and then the surface area filtration, leading to the changes in the filtration rate of the glomeruli[16].Gentamicin effect caused protein synthesis inhibition and DNA replication, because of the large oxidants accumulation in the kidney [17]. Many antioxidants which interfere with the Ros

production were used to change the nephropathic effect of gentamicin [18].

Histological examination of the gentamicin treated groups revealed remarkable renal damags [19,20,21]. The renal function test was affected after 5-7 days of gentamicin use. Gentamicin treated groups demonstrated critical increment in blood urea in the current study. The free radicals release from the renal tubules cell mitochondria was the main factor in the gentamicin – induced nephrotoxicity [22,23].

Aminoglycosides cause a simultaneous inhibition of variety of different membrane protein species including sodium/potassium- ATPase and release of lactate dehydrogenase, resulting in an apparently multifactorial cell death process [24] .Also it was found that aminoglycosides cause ATP depletion from either mitochondrial damage or direct inhibition of mitochondrial oxidative phosphorylation causing an oxidative injury [25]. Also renal tubular cells undergo necrosis when their cellular ATP stores are severely depleted to a level incompatible with maintenance of basal metabolism and activity of membrane transport pumps. [26]Results of this study showed an improvement in the serum creatinine and urea levels of Rabbits treated with combination of honey with gentamicin (group 3) compared with group 2, and these levels are near the levels in group1, and these results are in agreement with results of other study which showed that combination of cimetidine (an inhibitor of cytochrome P450) with gentamicin showed decrease in serum urea and creatinine levels. [27] The antioxidant effects of honey was attributed to its components, like antioxidant, trace elements and flavonoids compounds; therefore honey was suggested to be able to decrease lipid peroxidation.[28] Also the antioxidant activity of honey is due to the phenolic compounds and enzymes (glucose oxidase, catalase and peroxidase).[29,30] Also the content of L-ascorbic acid has a significant effect on total antioxidant activity of honey. [31]

The results of this study are in agreement with results of Heba *et al*, [32] which found that natural honey has protective effect against the damage in liver and kidney cells from oxidative stress induced by toxic level of lead in rats. Other study which found that co - administration of vitamins C and E significantly prevented the aminoglycosides - induced nephrotoxicity. [33]

Conclusion

This study showed that there was histological improvement in the kidney when honey given with gentamicin ,and also daily administration of honey could enhance the kidney functions and decrease the nephrotoxic effect induced by gentamicin.

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TJPS

تقييم الدور الوقائي لعسل الحمضيات citrus honey تجاه الاضرار النسجية المرضية والبيوحيوية للسمية الكلوية المستحثة بالجنتاميسين في الارانب المحلية *Oryctolagus cuniculus*

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الملخص

تعتبر السمية الكلوية المستحثة بالعقاقير من المسببات المهمة للفشل الكلوي. كما ان مضادات الامينوكلايكوسيد ومنها الجنتاميسين تسبب السمية الذاتية والسمية الكلوية كتأثير ثانوي رئيس, ولهذا تم التركيز على استخدام مواد طبيعية كمضادات اكسدة ضد الفعل المؤكسد السام الذي يسبب تأثير ضار للخلية. ومن اهم هذه المواد هو العسل. يهدف هذا العمل الى تقييم الدور الوقائي لعسل الحمضيات ضد الاضرار النسجية المرضية والبيوحيوية للسمية الكلوية المستحثة بالجنتاميسين. تم استعمال 24 ارنب مربى محليا (***Oryctolagus cuniculus***) مقسمة على 4 مجاميع (كل مجموعة مؤلفة من 6 ارانب), استلمت المجموعة الاولى جرعة يومية من المحلول الطبيعي IP (السيطرة) المجموعة الثانية استلمت (80ملغ/كغ/يوم) .IP من الجنتاميسين, المجموعة الثالثة استلمت (80ملغ/ كغ/يوم) .IP من الجنتاميسين مع جرعة فموية من عسل الحمضيات بجرعة 625 ملغ/كلغ/يوم, المجموعة الرابعة استلمت (80ملغ/ كغ/يوم) .IP من الجنتاميسين مع جرعة اضافية من العسل فمويا لمدة 14 يوم. كل الحيوانات (عند اليوم 15) تم تخديرها بالايثر وتم التضحية بها, ثم تم جمع عينات الدم للقياسات اللاحقة لكرياتنين المصل واليوريا والالبومين بينما عزلت الكلية في 10% من الفورمالدهايد لغرض الفحص النسجي المرضي. اظهرت هذه الدراسة ان الجنتاميسين يسبب سمية كلوية تمثلت بارتفاع مستوى الكرياتنين واليوريا في المصل وانخفاض مستوى بروتين الالبومين. بينما اختزل التأثير السمي في المجاميع المعاملة بالعسل والجنتاميسين وتمثل ذلك باختزال مستوى الكرياتنين واليوريا في المصل وارتفاع مستوى الالبومين وتحسن المؤشرات النسجية مقارنة مع المجموعة الثانية المعاملة بالجنتاميسين. استنتج من هذه الدراسة ان العسل اختزل السمية الكلوية المستحثة بالجنتاميسين.