Protective effect of citrus honey toward the histopathological and biochemical nephrotoxic damages of gentamicin–induced in local rabbits

Oryctolagus cuniculus

Riyam Ameen Salih¹, Abdul–Karim Salim Mahood², Samira Abdul-Hussain Abdulla³, Mohammed Abdullah Ajeel⁴

¹ Collage of Medicine, Wasit University, Wasit, Iraq
² Collage of Medicine, Tikrit University, Tikrit, Iraq
³ College of Pharmacy, Mosul University, Mosul, Iraq

DOI: http://dx.doi.org/10.25130/tjps.25.2020.001

ABSTRACT

Drugs induced nephrotoxicity now considered as an essential reason for kidney failure. Aminoglycoside anti-infection agents, for example, gentamicin, which causes ototoxicity and nephrotoxicity as a side effect, 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 antibacterials; 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]. Wojatk 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]

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-bacterials; they act especially against aerobic, gram-negative bacteria.[3] Gentamicin is one of the
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
**Group 1-** 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 -20°C 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.

**Group 2:** Gentamicin – treatment of adult rabbits causes vacuolation, hydroptic 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.

**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.
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  | 0.73±0.06        | 24±4.13    | 3.64±0.05     |
| X = SD   |                  |            |               |
| Group 2  | 0.93±0.10 *      | 45.8±4*    | 2.13±0.04*    |
| X = SD   |                  |            |               |
| Group 3  | 0.5±0.02*        | 37±3.5*    | 3.35±0.06     |
| X = SD   |                  |            |               |
| Group 4  | 0.72±0.028       | 38.75±3.7* | 4.4±0.21 *    |
| X = SD   |                  |            |               |

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

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]. Ali et al. [14] 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 damages [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 group 1, 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.

References

[1] Pannu, N. and Nadim, M. (2008). An overview of drug induced acute kidney injury. Crit. Care Med., 36 (4 suppl). S216-23.
[2] Marieb, E. (2006). Urinary system. In: Essentials of human anatomy and physiology. 8th ed., pbl. Pearson Benjamin Cummings, San Francisco, Boston, New York, London and Madrid ip. PP. 501-26.
[3] Leonard L., Liat V., and Mical P. (2009). Aminoglycoside drugs in clinical practic. Aminoglycoside drugs in clinical practic. 26. Boston, New York, London and Madrid ip.
[4] Langhendries J.P., Battisti O, Bertrand J.M. et al. (1993). Once-a-day administration of amikacin in neonates : assesment of nephrotoxicity and ototoxicity. Dev pharmacol Ther; 20: 220-30.
[5] Pedraza-Chaveri J, Maldonado PD, Medina-Campos O, et al. (2000). Garlic ameliorates gentamicin nephrotoxicity: Relation antioxidant enzymes. Exp physiology. 29:602-611.
[6] Wojciech, L., Vincent L. Pecoraro, and Jochen, S.t. (2005). Ternary Complexes of Gentamicin with Iron and Lipid Catalyze Formation of Reactive Oxygen Species. Chem. Res. Toxicol. 18 (2): 357.
[7] Carlos Martinez-Salgado a, et al(2007). Glomerular nephrotoxicity of aminoglycosides. Toxicology and Applied Pharmacology.; 223: 86–98.
[8] Dina, G. (2005). Natural Honey Q and A. Wordfeeder. Science of food and Agriculture.4:1-3.
[9] Ana C.S., Montserrat G., Cristina d. L. et al(2005). Estimation of the honeydew ratio in honey samples from their physicochemical data and from their volatile composition obtained by SPME and GC-MS. Science and Agriculture of food. 85: 817-824
[10] Saravanan K. J. and Mahitosh, M.(2009). Antiproliferative effect of honey and of polyphenols. Biomedicine and Biotechnology. 34: 1-10.
[11] Heinegard, D. and Tederstrom, G.(1973). Determination of serum creatinine by irect colorimetric method. Clin. Chem. Acta. 43:305-309.
[12] Fawcett, JK and Scott, JE .(1960). Determination of urea in blood or serum. J Clin. Path. 13: 156-159.
[13] Junqueira, L.C.; Carneiro, J. and Kelley, R. (1995). Basic Histology. 8th Ed, Lange Medical Book. 7: 1-2, 30G-314G.
[14] Kosek, J., Mazze, R. and Cousins, M. (1974). Nephrotoxicity of gentamicin. J. Lab. Invest. 30(1): 48-57.
[15] Parlakpinar, H., Koc, M., Polat, V., Ozer, M., Turkoz, Y. and (2004). Protective effect of aminoguanidine against nephrotoxicity induced by amikacin in rats. Urol Res. 22: 278-82.
[16] Baliga, R., Ueda, N., Walker, P. and Shah, S. (1999). Oxidant mechanisms in toxic acute renal failure. Drug Metab. Rev. 11:971-7.
[17] Sundin, D., Sandoval, R. and Molitoris, B. (2001). Gentamicin inhibits renal protein and phospholipid metabolism in rats: Implications
involving intracellular trafficking. J. Am. Soc. Nephrol. 12; 114-23.

[18] Nakajima, T., HItSIda, A., and Kato A. (1994). Mechanisms for protective effects of free radical scavengers on gentamicin mediated nephrotoxicity in rats. Am. J. Physiol. 266(3part2): 425-31.

[19] Abdel-Naim, A., Abdel-Wahab, M. and Attia, F. (1999). Protective effects of vitamin E and probucol against gentamicin –induced nephrotoxicity in rats. Pharmacol. Res. 40(2):183-187.

[20] Karahan I., Atessahin, A., Yilmaz, S., Ceribas, A. and Sakin, F. (2005). Protective effect of lycopene on gentamicin-induced nephrotoxicity oxidative stress and nephrotoxicity in rats. Toxicology. 215:198-204.

[21] Zeeni N., Selmaoui B., Beachamp, D., Labrecque, G. and Thibault, L. (2007). Dietary protein level alters gentamicin-induced nephrotoxicity in rats. Physiol. Behav. 90(5):760-770.

[22] Al-Majed A., Mostafa, A., Al-Rikabi, A. and Al-Shabanah, O. (2002). Protective effects of oral Arabic gum administration on gentamicin-induced nephrotoxicity in rats. Pharmacol. Research. 46(5):445-451.

[23] Abdel - Raheem, I., El-Sherbeny, G. and Taye, A. (2010). Green tea ameliorates of renal oxidative damage induced by gentamicin in rats. Pak. J. Pharm. Sci. 1(23):21-28.

[24] Marie, P. Mingeot, L. and Paul M. T.(1999). Aminoglycosides: Nephrotoxicity. Antimicrobial Agents and Chemotherapy. 43: 1003–1012.

[25] Joel D. K., Hu Ding, Annamaria L., Bela Li, David Pei-Yuan Qing, Lazlo D., et al.(2002). L-carnitin ameliorates gentamicin-induced renal injury in rats. Nephrol Dial Transplant. 17: 2122-2131.

[26] Wilfred, L., Saraha, M. and Jerrold S. Levine.(1998). Graded ATP depletion can cause necrosis or apoptosis of cultured mouse proximal tubular cells. Am J physical renal physiol. 274: 315-327.

[27] S. M. Poormosavl, M. A. Behmanesh and H Najafzaden.(2010). Effect of cimitidin on gentamicin-losartan induced nephrotoxicity in rats. Afr j Pharm. 4: 341-345.

[28] Sathyasurya, D. R.and Aziz Al-Safi I. (2009). Two varieties of honey that are available in Malaysia gave intermediate glycemic index values when tested among healthy individuals. Maced J Med Sci. 153(2): 145-148.

[29] Vilma, B., Petras, V., Violeta, C. (2007). Radical scavenging activity of different floral origion honey and bee bread phenolic extracts. Elsevier. 30: 1-6.

[30] Cristiane F. F., Alexandre K., Luciana M. K. et al. (2010). In vitro study of antioxidant and scavenger properties of phenolic compounds from lychnophora species. Maced J Med Sci. 33: 867-870.

[31] Aldina K.,Mazalović M., Aida C., et al.(2009). The influence of L-ascorbic acid content on total antioxidant activity of bee-honey. European Journal of Scientific Research. 32: 95-101.

[32] Heba M Halawa, Nagwa E El-Nefiawy, Noha A Makhlouf and Awatef A Mady.(2009). Evalution of honey protective effect on lead induced oxidative stress in rats. JASMR. 2: 197-209.

[33] Kadkhodae M., Khastar H., Faghihi M., et al.(2005). Effects of co-supplementation of vitamin E and C on gentamicin-induced nephrotoxicity in rats. Exp Physiol. 90: 571-576.
تقييم الدور الوقائي لعسل الحمضيات

للمسمية الكلوية المستحثة بالجنتاميسين في الأرانب المحلية

تريام أيمن صالح، عبد الكريم سالم ماهود، سميرة عبد الحسن، محمد عبد الله عجيل

1. كلية الطب، جامعة واسط، واسط، العراق
2. كلية الطب، جامعة تكريت، تكريت، العراق
3. كلية الصيدلة، جامعة الموصل، الموصل، العراق

تعتبر السمية الكلوية المستحثة بالعقاقير من المسببات المهمة لفشل الكمي. كما أن مضادات الأمينوكلايكوسيد ومنها الجنتاميسين تسبب السمية ذاتية والسمية الكموية كتأثير ثانوي رئيس، ولذا تم التركيز على استخدام مواد طبيعية كمضادات فعالة ضد الفعل المؤكسد السام الذي يسبب تأثير ضار للخلايا. ومن أهم هذه المواد هو العسل. يهدف هذا العمل إلى تقييم الدور الوقائي لعسل الحمضيات ضد الأضرار النسيجية السمية الكلوية والبيوحيوية للمسمية الكلوية المستحثة بالجنتاميسين. تم استعمال 24 أرنب مري محليا (Oryctolagus cuniculus) مقسمة إلى 4 مجموعات (كل مجموعة مقسمة إلى 6 أرانب). استعملت المجموعة الأولى جرعة يومية من المحلول الطبيعي IP (0.8 ملغ/كمغ/يوم) كمحمول طبيعي IP (السيطرة). المجموعة الثانية استطعت (0.8 ملغ/كمغ/يوم) IP من الجنتاميسين، المجموعة الثالثة استطعت (0.8 ملغ/كمغ/يوم) IP من الجنتاميسين مع جرعة اضافية من العسل فمويا لمدة 14 يوم. كل الحيوانات (بعد اليوم 15) تم تخديرها بالإيثر وتم التضحية بها. تم تجميع عينات الدم لقياسات الالبومين والكرياتين في المصل. توضح هذه الدراسة أن الجنتاميسين يسبب سمية كموية تمثل بارتفاع مستوى الكرياتين والكلي في المصل، وانخفاض مستوى الالبومين. بينما استخلص الدور الوقائي لعسل الحمضيات في المجموعة الستة بالعسل والجنتاميسين، وتمت تلك باختزال مستوى الكرياتين والكلي في المصل وارتفاع مستوى الالبومين، وتحسن المؤشرات السمية مقارنة بالجرعة الثانية. استنتج من هذه الدراسة أن العسل أختزل السمية الكلوية المستحثة بالجنتاميسين.