The Protective Effect of Honey Against Amikacin-induced Nephrotoxicity in Rats

Abeer R. Abd Ali* and Sajida H. Ismail**

*Ministry of Health, Iraq
**Department of Pharmacology and Toxicology, College of Pharmacy, University of Baghdad, Baghdad, Iraq

Abstract

Drug-induced nephrotoxicity is an important cause of renal failure. Aminoglycoside antibiotics, such as amikacin, which causes otoxicity and nephrotoxicity as a main side effects, this is focused on the use of natural materials as antioxidants against the toxic oxidative action that exert a cell damaging effect. The most important one of these materials is the honey. The aim of this work is to evaluate the antioxidative effects of honey against amikacin – induced nephrotoxicity. 18 albino rats divided into 3 groups (6 rats per each group), group 1 received I.P daily dose of normal saline (control), group 2 received (35 mg/kg/day) I.P dose of amikacin, and group 3 received (35mg/kg/day) of amikacin I.P dose in combination with oral dose of honey(500mg/kg/day) for 2 weeks. All animals (at 15th day) were anesthetized by ether and sacrificed; blood samples were collected for the subsequent measurement of the serum creatinine, urea, malondialdehyde (MDA) and glutathione (GSH) while an isolated kidney was kept in 10 % of formaldehyde for the histopathological examination. This study showed that amikacin causes nephrotoxicity represented by elevation of serum level of creatinine and urea, MDA and a decrease in the serum glutathione level. While the administration of honey in combination with amikacin reduced the nephro-toxic effect of amikacin that represented by a reduction of the serum creatinine and urea, MDA and elevation of glutathione levels with improvement of the kidney histological findings in comparison with group 2. This study concluded that, honey decreased nephrotoxicity induced by amikacin through interference with the oxidative stress process, i.e. honey acts as free radical scavenger.

Key words: amikacin, honey, nephrotoxicity, oxidative stress.

تأثير العسل كواقي للتسمم الكلوي المستحث بالآميكاسين

عبارة عبد علي* و ساحة حسن اسماعيل**، وزارة الصحة، العراق.

الخليفة

أن الآداب المستحثة للتسمم الكلوي تعتبر من أهم أسباب الفلك الكلوي ومن هذه الآداب المعضيات الحيوية مثل أميكاسين، والتي يمكن تسميتها ذاتي ولاكتات جلدية رئيسية، مما تستطيع استخدام المواد الطبيعية فmium لها الخصائص المعطاة للتسمم الكلوي ضد الجهد التاسمكي الذي يسبب الآميكاسين. ولهذه أن المواد المعطاة للتسمم الكلوي. يهدف من هذا البحث هو تقييم الآثار المعطاة للتسمم الكلوي ضد الجهد التاسمكي الذي يسبب الآميكاسين. اشتمل البحث على 18 من الجربون البيضاء مقسمة إلى 3 مجموعات (7 فئات في كل مجموعة)، المجموعة 3 نتائج جرعة من المغذي المالي في التجويف البيني (كجمع مع ومنطقة المجموعة الثالثة عولجت بمبلغ (35 ملغ/كغ/يوما) من الآميكاسين في التجويف البروتيوني، والمجموعة 3 عولجت بمبلغ (35 ملغ/كغ/يوما) من الآميكاسين في التجويف البريتيوني مقرونة مع جرعة صفرية من المغذي المالي (0 ملغ/كغ/يوما) لمدة أسبوعين. وبعد انتهاء هذه التجارب (في يوم 15 من المعاينة) تم تدحر جميع الحيوانات بالإثر ثم قثل الحيوانات وجمع عينات من الدم لقياس مستوى الكرتيتين والوريبرولين ميكوسيدب. جلوكانتون و (GSH) (الكرتيتين والوريبرولين وميكلوديدياهيد) والجدولواتين (MDA) (الكرتيتين والوريبرولين وميكلوديدياهيد) والجدولواتين (MDA) (الكرتيتين والوريبرولين وميكلوديدياهيد). أظهرت هذه الدراسة أن التأثير لأميكاسين (في المجموعة 3) تمثل بإرتفاع مستوى الكرتيتين والوريبرولين ميكلوديدياهيد وانخفاض في مستوى الجدولواتين في مصل الدم. مع أن أثار واضحة للنفط في النسيج الكلوي. في حين، حيث سبب المعاينة بالدم (مجمع 3) انخفاضاً معيناً في مستوى الكرتيتين والوريبرولين ميكلوديدياهيد مع ارتفاع (مصري في مسواة الجدولواتين في مصل الدم مع واضح في النتائج في النسيج الكلوي. تشير هذه النتائج إلى أن استخدام العسل مع الآميكاسين قليل يمكن وقوع من عملية التاسمك حيث عمل العسل على اقتصاص الجذور

الخليفة المفتاحية: عسل، آميكاسين، التسمم الكلوي

1 Corresponding author E-mail: ph.sajida@yahoo.com
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Introduction.
Renal cell injury may culminate in the cell death, which may occur through necrosis, apoptosis or other pathways. Chemicals in general can initiate toxicity because of their intrinsic reactivity with cellular macromolecules. They may require renal or extra renal bioactivation to reactive intermediate, or may initiate injury indirectly by inducing oxidative stress. Oxidative stress is caused by excessive production of reactive oxygen species and it may produce a major inter-related derangements of cellular metabolism, such as alteration of protein and nucleic acid structure, damage to DNA, induction of apoptosis, increase in intracellular free calcium, damage to membrane ion transport or destruction of the cells by lipid peroxidation. The antioxidants play major protective roles against the deleterious effects of oxidant agents produced in the human body. They include both enzymatic- (such as catalase, glutathione peroxidase and superoxide dismutase) and non enzymatic- substances (such as tocopherols, phenolic compounds, flavonoids, catechins, ascorbic acid and carotenoids).

Aminoglycosides are potent bactericidal antibiotics; they act particularly against aerobic, gram-negative bacteria. Amikacin is one of the aminoglycoside, mostly used for treatment of severe, hospital-acquired infections with multidrug resistant Gram negative bacteria such as Pseudomonas aeruginosa, Acinetobacter, and Enterobacter. Amikacin is not more and not less ototoxic or nephrotoxic than gentamicin, while Mingeot (1999) found that amikacin is less nephrotoxic than gentamicin. Aminoglycoside induced nephro and ototoxicity, which are the limiting factors for their clinical use, in which the oxygen free radicals have been involved. Wojekch Lesniak, et al (2005) found that aminoglycosides, exert their adverse renal effect by generation of reactive oxygen species. Additionally, it has been demonstrated that aminoglycoside form a complex with mitochondrial Fe²⁺ to catalyze the formation of free radicals.

Honey is sweet, thick syrup made by honey bees from nectar of flowers, the flowers from which bees gather nectar largely determine the color, flavor, and aroma of honey. It is basically a saturated water solution of sugar, which also includes a highly complex mixture of carbohydrates, enzymes, amino acids, organic acids, minerals, aromatic substances, pigments, wax, pollen. Studies reported that honey also possesses natural antioxidants through many compounds like vitamin C and polyphenols like chrys, pinobanksin, luteolin and pinocembrin that can decrease oxidative stress in humans. The aim of this study is to evaluates the possible protective effects of honey against nephrotoxicity induced by amikacin.

Materials and Method
18 Male albino strain rats with an average weight of (150-200g) were obtained from and maintained in the Animal House of the College of Pharmacy/ University of Baghdad under conditions of controlled temperature and humidity. The animals were fed commercial pellets and tap water. The honey used in this study was the eucalyptus honey, brought and produced by College of Agriculture/University of Baghdad. It was given to animals by oral gavages tube in a dose of 500mg/kg/day.

Experimental Protocol
Group 1- Six rats were treated with I.P injection of normal saline for 14 days. This group served as control.
Group 2– Six rats were treated with I.P injection of 35 mg/Kg/day of amikacin for 14 days. This group served as positive control for nephrotoxicity induced by amikacin.
Group 3- Six rats treated with oral dose of 500mg/kg/ day of honey concomitantly with I.P dose of amikacin (35mg/kg/ day) for 14 days. This group utilized to investigate the possible protective effect of honey against nephrotoxicity induced by amikacin.

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 obtained after the animals had been sacrificed. Samples were left to clot, and then centrifuged at 3000 rpm. for 15 minutes to separate serum, which was stored at -20°C until used for the determination of creatinine, urea, glutathione and MDA, while the kidney was kept in formaldehyde(10%) and utilized for histological examination using paraffin section technique. The possible histopathological changes were examined in the Teaching Laboratories of Baghdad Medical City. Statistical analysis was performed using unpaired Student’s t-test. Data were presented as mean± SD, P-values less than 0.05 were considered significant for all data obtained from this study.
Results

Nephrotoxic effects of amikacin

Effect of amikacin on the renal function tests

The results of this study showed significant increase (p<0.05) in the serum levels of both creatinine and urea of rats treated with 35 mg/kg/ day of amikacin (group 2) compared to the corresponding levels in the control animals (group 1); where serum levels for creatinine was (36.8±11.73 and 54.8±11.2) and that for urea was (7.22±4.5 and 8.13±0.9) in group 1 and 2, respectively. (Table 1, figure 1 and 2).

Effect of amikacin on the serum GSH and MDA levels:

This study showed significant decrease (p<0.05) in the serum level of GSH in rats treated with 35 mg/kg/ day of amikacin (group 2) compared to the corresponding level in the control animals (group 1). The serum levels of GSH were (2.638±0.742 and 1.598±0.566) in group 1 and 2, respectively; while there was significant increase (p<0.05) in the serum level of MDA in rats treated with 35 mg/kg/ day of amikacin (group 2) compared to the corresponding levels in the control animals (group 1). The serum levels of MDA were (4.14±1.4 and 6.18±1.007) in group 1 and 2, respectively. (Table 2, figure 2 and 3).

Effect of the combination of honey and amikacin on the kidney function test:

There were significant decrease (p<0.05) in the serum levels of both creatinine and urea of rats treated with 35 mg/kg/ day of amikacin + 500 mg/kg/day of honey (group 3) compared to the corresponding levels of rats treated with 35 mg/kg/ day of amikacin (group 2). Serum levels for creatinine were (54.8±11.2 and 42.8±7.5) and that for urea were (8.13±0.9 and 6.74±1.27) in group 2 and 3, respectively. (Table 3, figure 5 and figure 6).

Effect of the combination of honey with amikacin on the serum GSH and MDA levels:

There were significant increase (p<0.05) in the serum levels of GSH of rats treated with 35 mg/kg/ day of amikacin + 500 mg/kg/day of honey (group 3) compared to the corresponding levels of rats treated with 35 mg/kg/ day of amikacin (group 2) the serum levels of GSH were (1.598±0.566 and 3.68±0.887) in group 2 and 3, respectively; while there were significant decrease(p<0.05) in the serum levels of MDA in rats treated with 35 mg/kg/ day of amikacin + 500 mg/kg/day of honey (group3) compared to the corresponding levels of the rats treated with 35 mg/kg/ day of amikacin (group 2). The serum levels of MDA were (6.18±1.007 and 4.51±0.465) in group 2 and 3, respectively. (Table 4 and figure 8)

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Table 1: Effect of amikacin on the serum urea and creatinine (n=6)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group 1</th>
<th>Group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine mmol/liter</td>
<td>40.4±4.9</td>
<td>54.8±11.2*</td>
</tr>
<tr>
<td>Urea mmol/liter</td>
<td>7.2±0.45</td>
<td>8.13±0.9*</td>
</tr>
</tbody>
</table>

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

Table 2: Effect of amikacin on the serum MDA and GSH (n=6)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group 1</th>
<th>Group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSH µmol/L</td>
<td>2.638±0.742</td>
<td>1.598±0.566*</td>
</tr>
<tr>
<td>MDA µmol/L</td>
<td>4.14±1.4</td>
<td>6.18±1.007*</td>
</tr>
</tbody>
</table>

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

Table 3: Effect the combination of the honey with amikacin on the serum urea and creatinine (n=6)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group 2</th>
<th>Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine mmol/liter</td>
<td>54.8±11.2</td>
<td>42.8±7.5*</td>
</tr>
<tr>
<td>Urea mmol/liter</td>
<td>8.13±0.9</td>
<td>6.746±1.27*</td>
</tr>
</tbody>
</table>

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

Table 4: Effect the combination of the honey with amikacin on serum MDA and GSH (n=6)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group 2</th>
<th>Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSH µmol/L</td>
<td>1.598±0.566</td>
<td>3.68±0.887*</td>
</tr>
<tr>
<td>MDA µmol/L</td>
<td>6.18±1.007</td>
<td>4.51±0.465*</td>
</tr>
</tbody>
</table>

X=mean SD=standard deviation, n=number of animals, * =significant (p<0.05)
The protective effect of honey against amikacin

Figure 1: Effect of Amikacin on the serum urea

Figure 2: Effect of amikacin on the serum creatinine

Figure 3: Effect of amikacin on the serum MDA

Figure 4: Effect of amikacin on the serum GSH

Figure 5: Effect of the combination of honey with amikacin on the serum urea

Figure 6: Effect combination of the honey with amikacin on serum creatinine
Effects of combination of honey with amikacin on the histology of the kidney.

Kidneys of group 1 (control) showed that the glomeruli consist of tuft of capillaries surrounded by Bowman's capsule. The renal tubules have a normal appearance figure (9), the structure consist of proximal convoluted tubule in which lined by columnar epithelial cell and small lumen while, the distal convoluted tubule lined by occupied cell with large luminal. The collecting tubule appear large in diameter and surrounded by occupied epithelial cells. (Figure 10) Kidney of group 2 (rats treated with amikacin) showed a marked shrinkage of glomeruli structure with degeneration and necrosis of renal Kidneys of group 3 (rats treated with amikacin and honey) showed a mild degenerative changes of the renal tubules (proximal, distal and the collecting duct). While, there were regenerative changes of some renal tubules especially proximal tubules and columnar lining epithelial with an improvement of approximately 70% compared to the group 2 animals, and the appearance was look like the control group. (Figure 11)
The protective effect of honey against amikacin

Discussion

Aminoglycoside antibiotics have long been used as antibacterial therapy. Despite their beneficial effects, aminoglycosides have considerable ototoxic and nephrotoxic side effects (18). It has been reported that amikacin may induce free radical production which implicates a variety of pathological processes (19,20). In this study the marked elevation of the levels of both serum creatinine and urea in group 2 compared with group 1 were observed and give an indication to the reduction in the glomerular filtra
tion. Since serum creatinine and urea are waste products of protein metabolism that need to be excreted by the kidney; therefore such increase of serum creatinine and urea as reported in this study confirm an indication of functional damage of the kidney and these results were in consistent with other studies (18,21). The nephrotoxicity of aminoglycoside (represented by acute tubular necrosis) usually appeared 5 to 10 days after a toxic insult and may be seen even after discontinuation of aminoglycosides therapy. The elevation of the serum creatinine and urea may be seen in association with hypomagnesemia and hypokalemia. In general, aminoglycosides induced acute kidney injury results in non oliguric renal failure (11,19,22). Moreover, the results of this study showed a significant decrease in the serum levels of GSH with significant increase in the contents of the end product of lipid peroxidation (MDA) of group 2 compared with group 1. Previous studies showed that in the amikacin treated rats there were had a significant increase in the serum level of MDA, suggesting the involvement of oxidative stress in the nephrotoxicity. (21,23) Kamil et al (2008) showed that MDA is one of the well-known secondary products generated after exposure to reactive oxygen species and free radicals, and it may be used to evaluate oxidative damage by measuring serum levels of thiobarbituric acid reactive substance (24). Glutathione (GSH) is a one of the natural antioxidant that protect cells from free radical toxins, it is found exclusively in its reduced form, since the enzyme that reverses it from its oxidized (GSSG) to reduced form is constitutively active and inducible upon oxidative stress (25). Therefore GSH is an important naturally occurring antioxidant and its level in the tissue is considered a critical determinant of the threshold for tissue injury, and an explanation for decreased GSH after amikacin treatment to the increased consumption of GSH in non-enzymatic and enzymatic removal of oxygen radicals with efflux of GSSG being the major factor responsible for maintenance of redox ratio (26). Concerning the results of this study which showed amikacin nephrotoxic effects is in agreement with similar other study in which a significant depletion of GSH in kidney cells resulting in their damage due to enhancement of lipid peroxidation. (27) Aminoglycoside antibiotics are known to be transported and accumulated within lysosomes of renal proximal tubular cells and to causes proximal tubular cell injury and necrosis. The pathogenesis of aminoglycoside nephrotoxicity is postulated to be related to the capacity of these organic polycations to interact electrostatically with membrane anionic phospholipids and to disrupt membrane structure and function (28). It was demonstrated that lipid peroxidation moieties like O2-, hydrogen peroxide (H2O2) and hydroxyl radicals were increased with aminoglycoside therapy (29,30). In addition, the results of this

Figure 11: Sections of kidney show slightly degenerative changes with regeneration of renal tubule in which the appearance look like the normal in amikacin with honey treated group.

Blue arrow represents glomeruli.
Red arrow represents regeneration of PCTs.
White arrow represents regeneration of DCTs.
Magnification: (100 X); Staining: Haematoxylline and Eosin.
Conclusion

This study showed that daily administration of honey was able to improve the renal functions (creatinine and urea) and decrease the nephrotoxicity induced by 35 mg/kg daily I.P dose of Amikacin, through interference with the oxidative stress process (MDA and GSH), also there is histological improvement in the kidney when honey given with amikacin, i.e. honey acts as a free radical scavenger.

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