

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

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### Abstract

Drug –induced nephrotoxicity is an important cause of renal failure. Aminoglycoside antibiotics, such as amikacin, which causes ototoxicity 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 antioxidant 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 15<sup>th</sup> day) were anesthetized by ether and sacrificed; blood samples were collected for the subsequent measurement of the serum creatinine, urea, malneldehyde (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 كواقى للتسمم الكلوي المستحث بالأميكاسين عبر رياض عبد علي\* و ساجدة حسين اسماعيل\*\*,1

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### الخلاصة

ان الادوية المستحثة للتسمم الكلوي تعتبر من اهم اسباب الفشل الكلوي ,ومن هذه الادوية المضادات الحيوية مثل أمينوكليكو سيد ، والذي يسبب تسميم أذني وكلوي كآثار جانبية رئيسية، مما تستدعي استخدام المواد الطبيعية ممن لها الخاصية المضادة للتأكسد للعمل ضد الجهد التأكسدي التي يسببه الاميكاسين . واحد من أهم هذه المواد المضادة للتأكسد هو العسل. الهدف من هذا البحث هو تقييم الآثار المضادة للتأكسد للعسل ضد التسمم الكلوي الذي يسببه الأميكاسين . اشتمل البحث على ١٨ من الجرذان البيضاء مقسمة إلى ٣ مجاميع (٦ فئران في كل مجموعة)، المجموعة ١ تلقت جرعة من المغذي المالح في التجويف البطني (كمجموعه سيطرة)، المجموعة الثانية عولجت ب (٣٥ ملغ / كغ / يوميا) من الاميكاسين في التجويف البريتوني ، والمجموعة ٣ عولجت ب(٣٥ملغ/كغ/يووميا) من الأميكاسين في التجويف البريتوني مع جرعة فموية من العسل (٥٠٠ مغ/كغ/يووميا) لمدة اسبوعين. وبعد انتهاء مدة العلاج (في يوم ١٥ من المعالجة ) تم تخدير جميع الحيوانات بالايثر ثم قتل الحيوانات و جمع عينات من الدم لقياس مستوى الكرياتينين واليوربا و MDA (المالونديالدهايد ) والجلوتاثيون (GSH) في مصل الدم بينما حفظ نموذج من الكلى في ١٠٪ من الفورمالديهايد وذلك لفحص النسيج الكلوي . أظهرت هذه الدراسة أن التأثير الكلوي للاميكاسين (في المجموعة ٢) تمثل بارتفاع مستوى الكرياتينين و اليوربا و المالونديالدهايد وانخفاض في مستوى الجلوتاثيون في مصل الدم مع آثار واضحة للتلف في النسيج الكلوي . في حين سببت المعالجة بالعسل (مجموعة ٣) انخفاضاً معنوياً في مستوى الكرياتينين واليوربا والمالونديالدهايد مع ارتفاع معنوي في مستويات الجلوتاثيون في مصل الدم . مع تحسن واضح في نتائج الفحص الكلوي النسيجي مقارنة مع دراسة مجموعة ٢ . خلصت الدراسة إلى أن استخدام العسل مع الأميكاسين قلل وبشكل واضح من عملية التأكسد حيث عمل العسل على اقتناص الجذور الحرة الراديكالية. مما وفر حماية للكليتين من التأثير السمي للاميكاسين .

الكلمات المفتاحية : عسل ، اميكاسين ، التسمم الكلوي .

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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.<sup>(1)</sup> 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.<sup>(2)</sup> 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).<sup>(3)</sup> Aminoglycosides are potent bactericidal antibiotics; they act particularly against aerobic, gram-negative bacteria.<sup>(4)</sup> 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,<sup>(5)</sup> while Mingeot(1999) found that amikacin is less nephrotoxic than gentamicin.<sup>(6)</sup> Aminoglycoside induced nephro and ototoxicity, which are the limiting factors for their clinical use, in which the oxygen free radicals have been involved.<sup>(7)</sup> Wojckch Lesniak, *et al* (2005) found that aminoglycosides, exert their adverse renal effect by generation of reactive oxygen species.<sup>(8)</sup> Additionally, it has been demonstrated that aminoglycoside form a complex with mitochondrial Fe<sup>+2</sup> to catalyze the formation of free radicals.<sup>(9)</sup> 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<sup>(10)</sup>. 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.<sup>(11)</sup> 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.<sup>(7, 11,12)</sup> The aim of this study is to evaluate 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 gavage tube in a dose of 500mg/kg/day.<sup>(11)</sup>

## 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<sup>(13)</sup>, urea<sup>(14)</sup>, glutathione<sup>(15)</sup> and MDA<sup>(16)</sup>; while the kidney was kept in formaldehyde(10%) and utilized for histological examination using paraffin section technique.<sup>(17)</sup> 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 ( $36.8 \pm 11.73$  and  $54.8 \pm 11.2$ ) and that for urea was ( $7.22 \pm 4.5$  and  $8.13 \pm 0.9$ ) 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 ( $2.638 \pm 0.742$  and  $1.598 \pm 0.566$ ) 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 ( $4.14 \pm 1.4$  and  $6.18 \pm 1.007$ ) 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 ( $54.8 \pm 11.2$  and  $42.8 \pm 7.5$ ) and that for urea were ( $8.13 \pm 0.9$  and  $6.746 \pm 1.27$ ) 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 ( $1.598 \pm 0.566$  and  $3.683 \pm 0.887$ ) 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 (group 3) compared to the corresponding levels of the rats treated with 35

mg/kg/ day of amikacin (group 2). The serum levels of MDA were ( $6.18 \pm 1.007$  and  $4.51 \pm 0.465$ ) in group 2 and 3, respectively. (Table 4 and figure 8)

**Table1: Effect of amikacin on the serum urea and creatinine (n=6)**

Parameter	Group1 X ± SD	Group2 X±SD
Creatinine mmol/liter	40.4±4.9	54.8±11.2*
Urea mmol/liter	7.2±0.45	8.13±0.9*

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)**

Parameters	Group1 X±SD	Group2 X±SD
GSH $\mu\text{mol/L}$	2.638±0.742	1.598±0.566*
MDA $\mu\text{mol/L}$	4.14±1.4	6.18 ±1.007

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)**

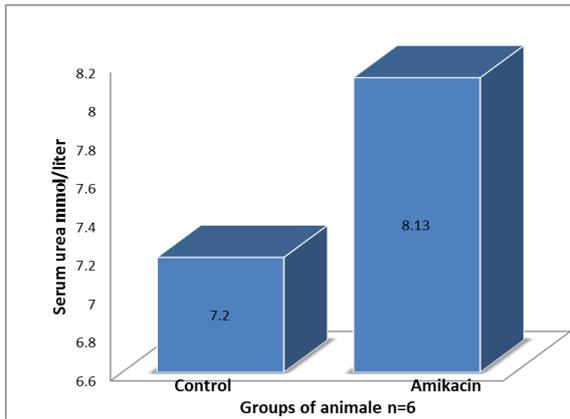
Parameter	Group 2 X±SD	Group 3 X±SD
Creatinine mmol/liter	54.8±11.2	42.8±7.5*
Urea mmol/liter	8.13±0.9	6.746±1.27*

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

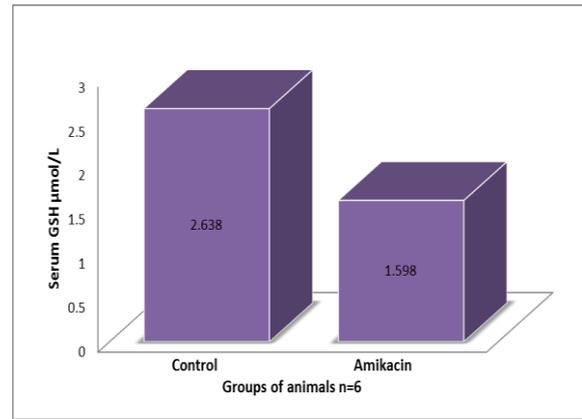
**Table 4: Effect the combination of the honey with amikacin on serum MDA and GSH (n=6)**

Parameters	Group2 X±SD	Group`3 X±SD
GSH $\mu\text{mol/L}$	1.598±0.566	3.683±0.887*
MDA $\mu\text{mol/L}$	6.18±1.007	4.51±0.465*

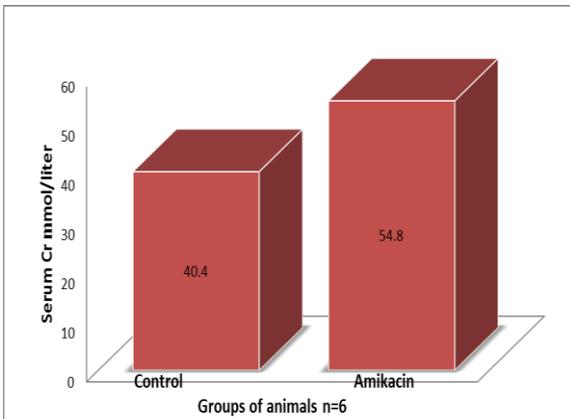
X=mean SD=standard deviation, n=number of animals, \* =significant ( $p < 0.05$ )



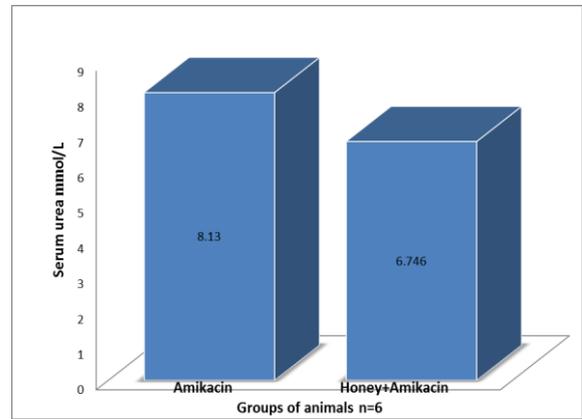
**Figure 1: Effect of Amikacin on the serum urea**



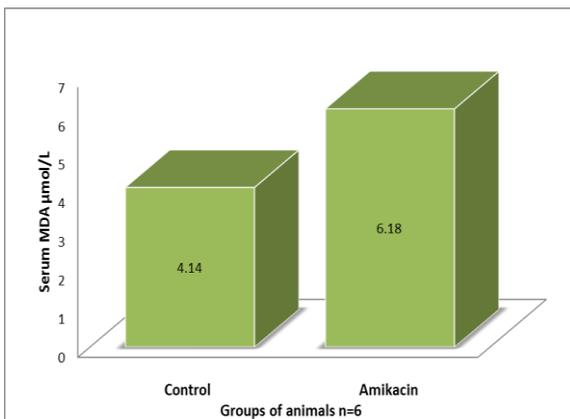
**Figure 4: Effect of amikacin on the serum GSH**



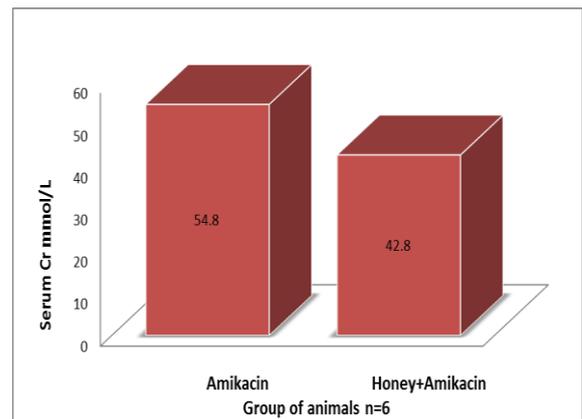
**Figure 2: Effect of amikacin on the serum creatinine**



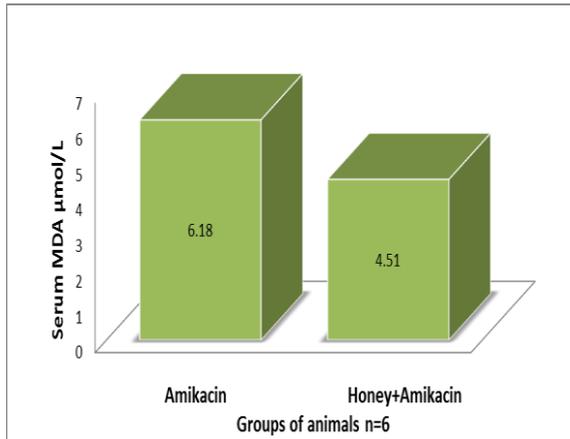
**Figure 5: Effect of the combination of honey with amikacin on the serum urea**



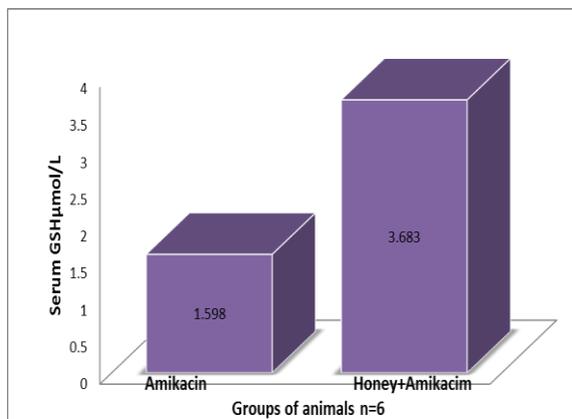
**Figure 3: Effect of amikacin on the serum MDA**



**Figure 6: Effect combination of the honey with amikacin on serum creatinine**



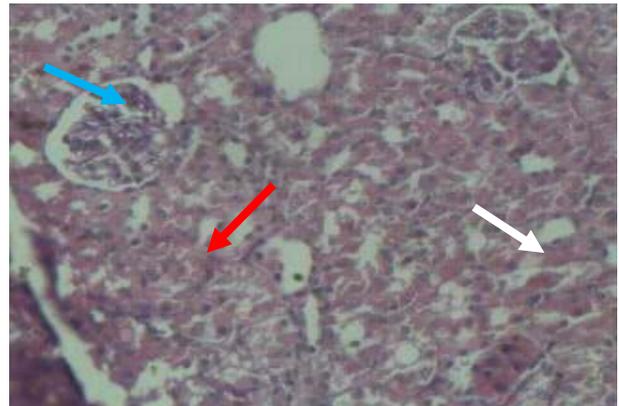
**Figure 7:** Effect combination of the honey with amikacin on the serum MDA



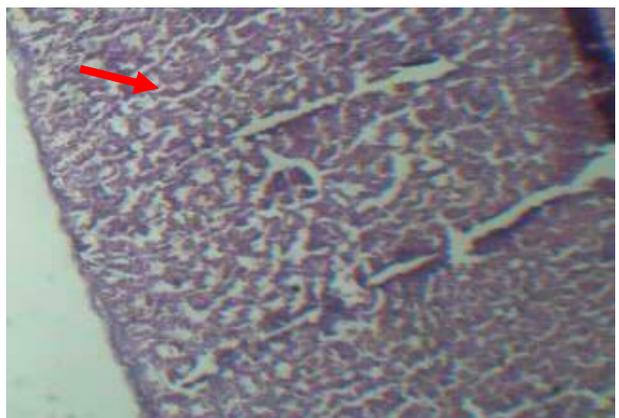
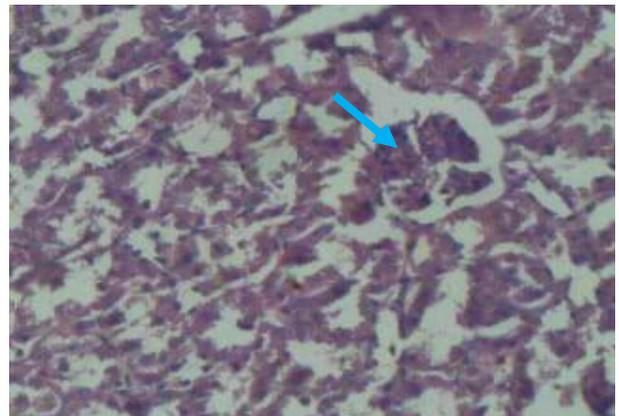
**Figure8:** Effect of combination of the honey with amikacin on the level of serum GSH

**Effects of combination of honey with amikacin on the histology of the kidney.**

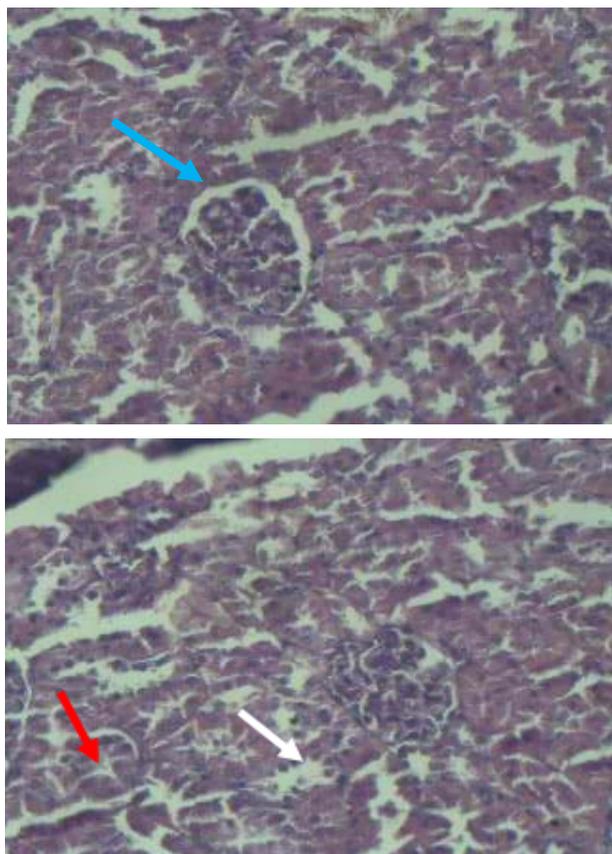
Kidneys of group 1 (control) showed that the glomeruli consist of tuft of capillaries surrounded by Bowman's capsule. The renal tubules have a normal appearance figure(9), the structure consist of proximal convoluted tubule in which lined by columnar epithelial cell and small lumen while, the distal 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)



**Figure 9 :** Section of the kidney show normal structure appearance of the glomerulai and renal tubules of control group. **Blue arrow** represents the glomerulus. **Red arrow** represents the PCTs. **White arrow** represents the DCTs. **Magnification:** (100 X); Staining: Haematoxylline and Eosin.



**Figure :10** Sections of kidney show degenerative changes and necrosis of renal tubules in amikacin treated group. **Blue arrow** represents shrinkage of glomerular **Red arrow** represents necrosis of PCT **Magnification:** (100 X); Staining: Haematoxylline and Eosin.



**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 treted group.**

**Blue arrow** represents glomeruli.

**Red arrow** represents regeneration of PCTs.

**White arrow** represents regeneration of DCTs.

**Magnification:** (100 X); Staining:

Haematoxylline and Eosin.

## Discussion

Aminoglycoside antibiotics have long been used as antibacterial therapy. Despite their beneficial effects, aminoglycosides have considerable oto and nephro-toxic side effects<sup>(18)</sup>. It has been reported that amikacin may induce free radical production which implicates a variety of pathological processes.<sup>(19,20)</sup> 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 filtration. 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<sup>(18,21)</sup>. 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<sup>(11,19,22)</sup> 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.<sup>(21,23)</sup> 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.<sup>(24)</sup> 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 reveres it from its oxidized (GSSG) to reduced form is constitutively active and inducible upon oxidative stress<sup>(25)</sup>, 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<sup>(26)</sup>, 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.<sup>(27)</sup> 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.<sup>(28)</sup> It was demonstrated that Lipid peroxidation moieties like O<sup>·</sup>, hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and hydroxyl radicals were increased with aminoglycoside therapy.<sup>(29,30)</sup> In addition, the results of this

study showed a marked shrinkage of glomeruli structure in kidneys of rats in group 2 with the appearance of degeneration and necrosis in the renal epithelial cells in the proximal, distal and the collecting duct unlike the structure of the kidneys from other groups of animals. 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.<sup>(31)</sup> Also it was found that aminoglycosides cause ATP depletion from either mitochondrial damage or direct inhibition of mitochondrial oxidative phosphorylation causing an oxidative injury.<sup>(32)</sup> 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.<sup>(33)</sup> Results of this study showed an improvement in the serum creatinine and urea levels of rats treated with combination of honey with amikacin (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.<sup>(34)</sup> The elevation of GSH levels in the serum of group 3, and decrease of MDA levels in comparison with group 2, attributed to the free-radical scavenging properties of the honey where it help in maintaining the levels of reduce glutathione and MDA.<sup>(35)</sup> The antioxidant effects of honey was attributed to its constituents like antioxidant trace elements and flavonoids compounds; therefore honey has been suggested to be able to decrease lipid peroxidation.<sup>(36)</sup> Also the antioxidant activity of honey is due to phenolic compounds and enzymes (glucose oxidase, catalase and peroxidase).<sup>(37,38)</sup> Also the content of L-ascorbic acid has a significant impact on total antioxidant activity of honey.<sup>(39)</sup> Results of this study are in agreement with results of Heba M Halawa, *et al* (2009), 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.<sup>(40)</sup> And other study which found that co-administration of vitamins C and E significantly prevented the aminoglycosides-induced nephrotoxicity demonstrated by preservation of GFR and GSH levels and prevention of the elevation of urinary enzyme activities.<sup>(41)</sup>

## 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 35mg/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 free radical scavenger.

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