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ORIGINAL ARTICLE

THE PROTECTIVE EFFECT OF BETA CAROTENE AGAINST AMIKACIN INDUCED NEPHROTOXICITY

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Summary

Background: The rapid loss of kidney function is known as nephrotoxicity. The harmful effects of medications may cause this. Amikacin-related nephrotoxicity is an excellent illustration of such a case. Amikacin is a synthetic aminoglycoside that works against the majority of gram-negative bacteria and, in some circumstances, can be harmful to the kidneys. One of the many methods used to prevent or decrease this toxicity is the use of antioxidant substances with amikacin. Beta carotene is an antioxidant carotenoid pigment.
The aim of the current study: is to illustrate the ameliorative effect of beta carotene against the nephrotoxicity caused by amikacin when given before or during the treatment of amikacin. The study also evaluates the nephrotoxicity evoked by amikacin.
Material and Methods: Five groups of animals were used (seven rats in each group): the control group, the amikacin group, the beta carotene group, the beta carotene with the amikacin group, and the group given beta carotene first, then added amikacin later together with the beta carotene.
Results: The results of the current study demonstrate that when comparing the amikacin-treated group to the control group, there was a significant elevation ($p < 0.05$) of serum creatinine and urea levels. The results also showed that when comparing the amikacin -treated group to the group treated with beta carotene during amikacin treatment for 14 days, there was a significant reduction ($p < 0.05$) in serum creatinine and urea levels. Furthermore, in comparison of the amikacin -treated group to the group treated with beta carotene only for 9 days and continued with amikacin for 14 days, there was a significant reduction ($p < 0.05$) in serum creatinine and urea levels. Also, there were significant decreases (near normal) in serum creatinine and urea. Histological findings confirmed these findings; there was damage to renal tissue in the amikacin -treated group, whereas groups treated with beta carotene and amikacin showed improvements in histological images.
Discussion and conclusion: According to the results of renal function tests and histological findings, treatment with AMK only causes renal toxicity in rats, and when rats are treated with BC before and during AMK treatment or even only during AMK treatment, BC can prevent this renal toxicity by decreasing serum urea and creatinine levels, which, in agreement with the previous study, leads us to conclude that BC offers significant protection against AMK-induced nephrotoxicity, The results also showed the efficiency of two methods of administration of beta carotene in giving the required protection against renal toxicity (not preferring one method over the other).

Key words: Amikacin; Beta carotene; Creatinine; Nephrotoxicity; Urea

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Introduction

The kidney is an important organ for our body. It plays a vital role in homeostasis because it regulates the elimination of metabolic wastes as well as the control of acid-base balance, electrolyte concentration, and extracellular fluid volume regulation (1). Aminoglycosides (AMGs) can cause renal toxicity as one of their major adverse effects, and this makes their use low. The mechanism of nephrotoxicity comes from the accumulation of AMGs in the cortex of the kidney, which evokes the production of free radicals, results in oxidative stress, causes damage to glomerular tubules, and finally results in nephrotoxicity (2).

Amikacin (AMK), a semisynthetic AMG, has a broad bacterial spectrum of activity with low resistance from bacteria. Like other AMGs, it is a bactericidal antibiotic. It binds to the A site of 16S ribosomal RNA of the 30S ribosome, thus inhibiting protein synthesis, which leads to bacterial cell damage (3). For maximum effect of AMK, it should be given in a high concentration because its efficacy is dose-dependent and this requires close monitoring of the plasma concentration because of its narrow therapeutic index (4). Beta carotene (BC) is a tetraterpenoid carotenoid compound present in fruits and vegetables as a pigment (5), as well as being present in human milk and colostrum (6). Many benefits of BC are discovered, one of which is the antioxidation, anticancer effect, and eye health. In addition to that, vitamin A can be gained from BC (7, 8).

Animal Used in the Study

Thirty-five male albino rats, with an average weight of 200–250 g, were in each group. They are obtained from the animal house in the College of Veterinary Medicine-University of Mosul under conditions of controlled temperature. The animals were fed commercial pellets.

Experimental Protocol

1. **Control group:** For 23 days, the rats in the control group drank distilled water.

2. **AMK group:** The rats in this group received 150 mg/kg/day of AMK intraperitoneally for two weeks (9, 10).

3. **BC group:** The rats in this group received 100 mg/kg/day of BC orally for 9 days (11, 12).

4. **BC + AMK group:** The rats in this group received 150 mg/kg/day of AMK intraperitoneally and, at the same time, received 100 mg/kg/day of BC orally for two weeks.

5. **BC → (BC + AMK) group:** The rats in this group received 100mg/kg/day of BC orally for 9 days. After that, the same group E received 150 mg/kg/day of AMK intraperitoneally and, at the same time, received 100 mg/kg/day of BC orally for two weeks (11, 13, 14).

Materials and Methods

Materials

The BC used in this study was imported from Turkey and manufactured by PHARMAROYA company®- Turkey.

The AMK 500 mg used in this study was obtained from local pharmacies in Iraq and manufactured by MedoChemie®-Cyprus.

Methods

Firstly, the rats were euthanized by anesthetic ether. The sample was prepared and put in a plain tube after collecting the blood from the neck, and then, by using a glass rod to disperse the clot at 3000 rpm for 15 minutes, it was centrifuged. The storage of serum was at -20º C until used for the laboratory assays.

Biochemical Assays

1. Determination of serum urea

The spectrophotometry procedure is used in the current study for the detection of urea, which can be produced by several reactions, and the product carries a colored complex that was evaluated in the kit. The "Biosystem URE/BUN–COLOR UREASE/SALICYLATE kit" was used in this test (Spain).

2. Determination of serum creatinine

For the detection of creatinine, a colorimetric reaction between creatinine and alkaline picrate was applied according to the Jaffe technique principle, and the determination of creatinine was at 490 nm (490–510) in a kinetic manner, with no treatment step discovered. The amount of creatinine in the samples determines the rate of dye production. The presence of an initial rate method contributes to an improvement in reaction adaptability, speed, and specificity. This test used the "BIOLABO CREATININE Kinetic method" kit (France).

Histological Determination

Kidneys were removed from each rat and divided into the lobes after cleaning. The lobes were fixed in 10% neutral buffered formalin and embedded in paraffin. The paraffin-embedded blocks were cut to 4–5 µm and stained with hematoxyline and eosin (H-E). The slides were then examined using a light microscope (Olympus BX50) and photographed. All histological evaluations were made twice under blind conditions (without knowledge of the treatment).

Statistical Analysis

The serum urea and creatinine were analyzed by one-way ANOVA. Post-hoc comparisons were done using Tukey's tests using the statistical program GraphPad Prism V.9. Differences were considered significant at $p < 0.05$. Results are expressed as mean ±SD.

Results

Biochemical Results

Serum urea level is significantly increased in AMK-treated group compared to control group (control: 54.30 mg/dl±6.10 vs. AMK: 142.0±52.45), while coadminstration of BC with AMK group showed significantly drop in the elevation of serum urea level copmared to AMK-treated group (62.49 mg/dl±13.98), furthermore administration of BC before and during the treatment of AMK group, also demonstrate decreasing in elevated serum urea level compared with AMK-treated group (85.57 mg/dl±30.74). As seen in figure (1).

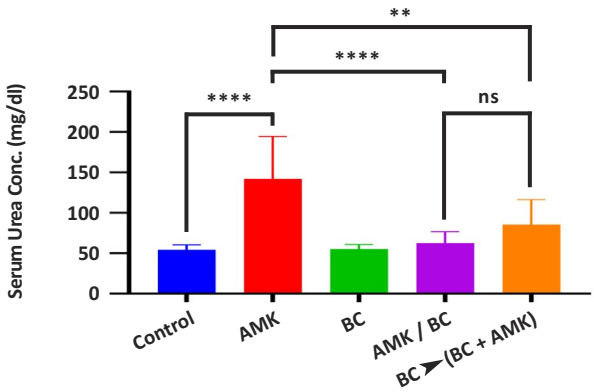


Figure 1: The modulating effect of BC and AMK on serum urea level. The data are presented as mean ± S.D. * indicates $p < 0.05$, ** indicates $p < 0.01$, **** indicates $p < 0.0001$, and ns indicates non-significant difference. One-way ANOVA was used, followed by Tukey's post hoc test against control values.

Serum creatinine level is significantly elevated in the AMK-treated group compared to the control group (control: 0.05 mg/dl±6.10 vs. AMK: 1.114±0.24), whereas the coadministration of BC with AMK reduced the elevation of serum creatinine level in comparison to the AMK-treated group (62.49 mg/dl 13.98). In addition, administration of BC before to and during treatment with AMK led to a reduction in high blood creatinine levels compared to the AMK-treated group (85.57 mg/dl 30.74 mg/dl). As seen in figure (2).

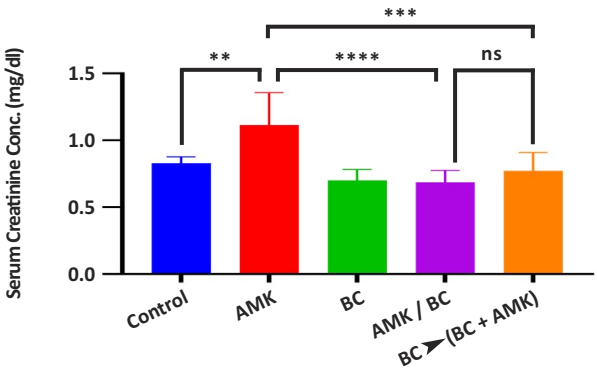


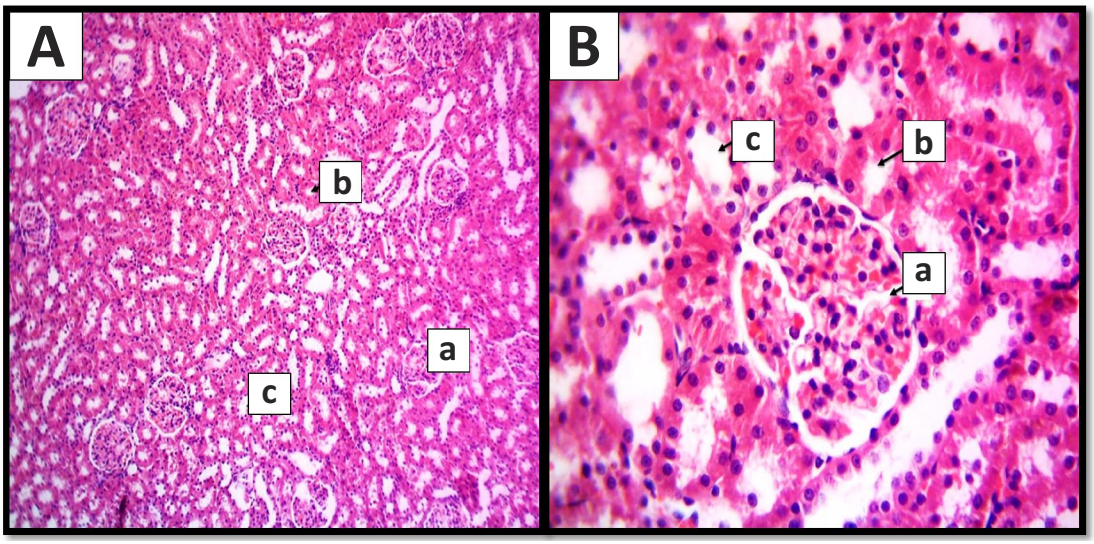
Figure 2: : The modulating effect of BC and AMK on serum creatinine level. The data are presented as mean ± S.D. * indicates $p < 0.05$, ** indicates $p < 0.01$, *** indicates $p < 0.001$, **** indicates $p < 0.0001$, and ns indicates non-significant difference. One-way ANOVA was used, followed by Tukey's post hoc test against control values.

Serum urea and creatinine levels showed a non-significant difference between groups treated with BC with AMK in different ways. (Figures 1-2).

2. Histological Results

Control group: (treated with D.W throughout the study)

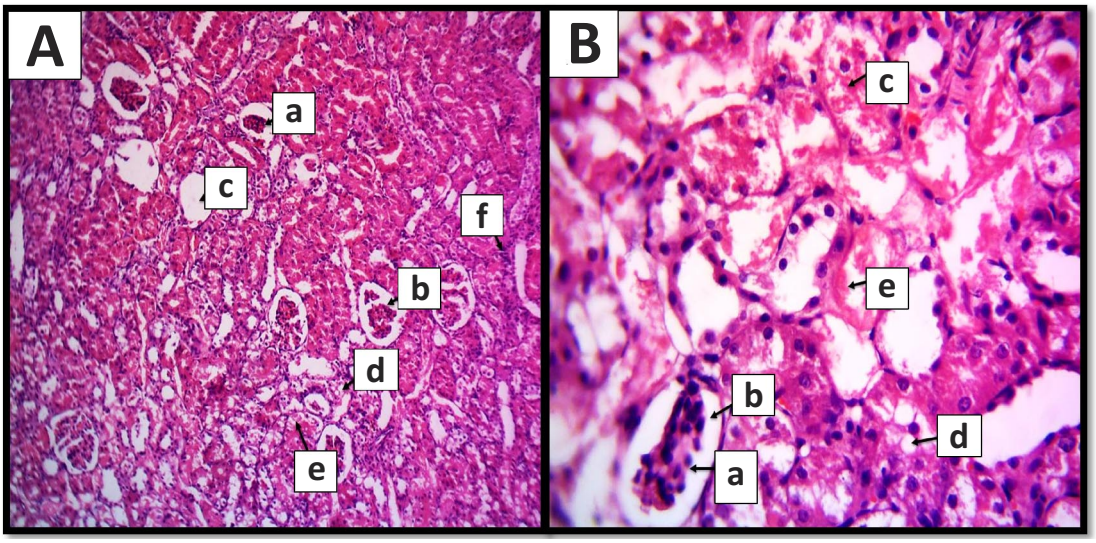
Light microscopic study show normal architecture of renal tissue characterized by normal glomeruli, proximal renal tubules, distal renal tubules no changes noted (-ve) and normal Bowman's space (Figures 3) (Table 1).



Figures 3. Photomicrograph of rat kidney of control group shows normal architecture of renal tissue characterized by glomeruli (a), proximal renal tubules (b) and distal renal tubules (c); A (100X), B (400X), H&E.

AMK group: (Treated with AMK for 14 days)

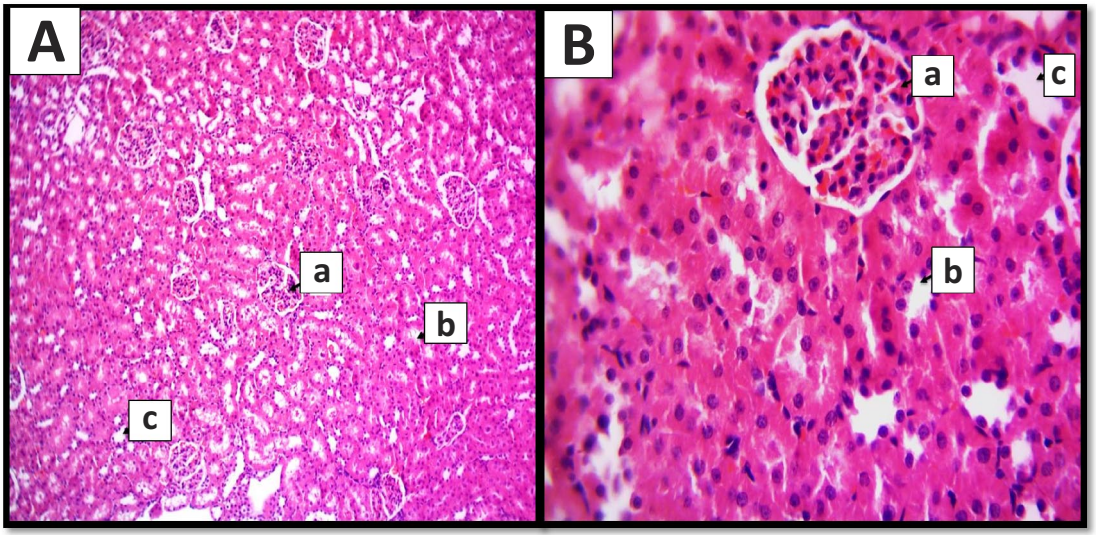
The morphological pictures of the renal tissues for AMK alone treated rats showed sever atrophy of glomeruli, dilatation of Bowman's space, renal cyst, degeneration and sever necrosis of epithelial cells lining renal tubules, with sever infiltration of inflammatory cells, vacuolar degeneration and severe cloudy swelling (Figures 4) (Table 1).



Figures 4. Photomicrograph of rat kidney of AMK group shows atrophy of glomeruli (a), dilatation of Bowman's space (b), renal cyst (c), degeneration (d) and necrosis (e) of epithelial cells lining renal tubules and infiltration of inflammatory cells (f); A (100X), B (400X), H&E stain.

BC group: (Treated with BC for 9 days)

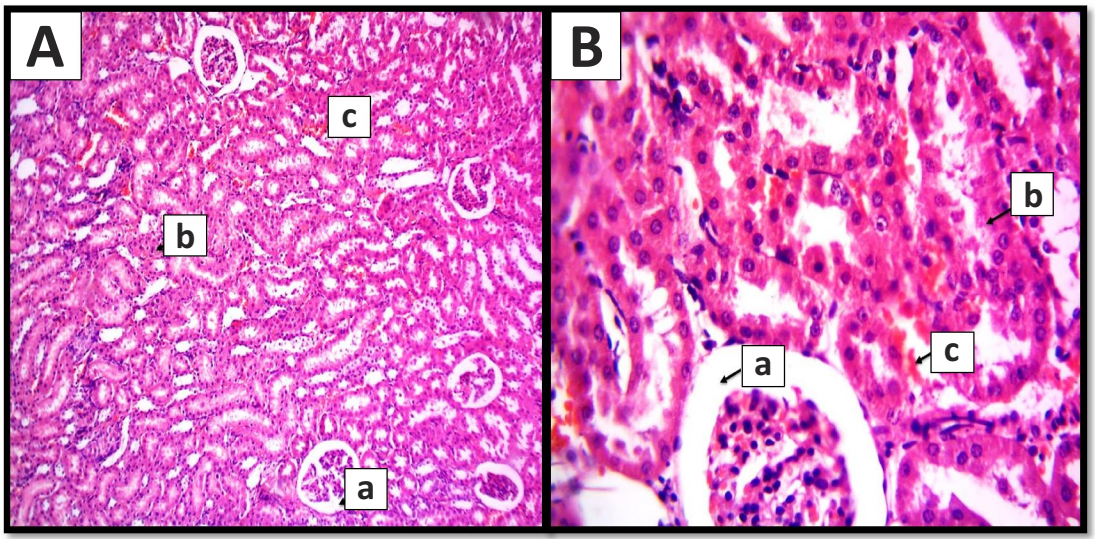
The effect of BC alone showed normal histological findings revealed normal architecture of renal tissue characterized by glomeruli, proximal renal tubules and distal renal tubules (Figures 5) (Table 1).



Figures 5. Photomicrograph of rat kidney of BC group shows normal architecture of renal tissue characterized by glomeruli (a), proximal renal tubules (b) and distal renal tubules (c); A (100X), B (400X), H&E stain.

BC+ AMK group: (Treated with BC and AMK together for 14 days)

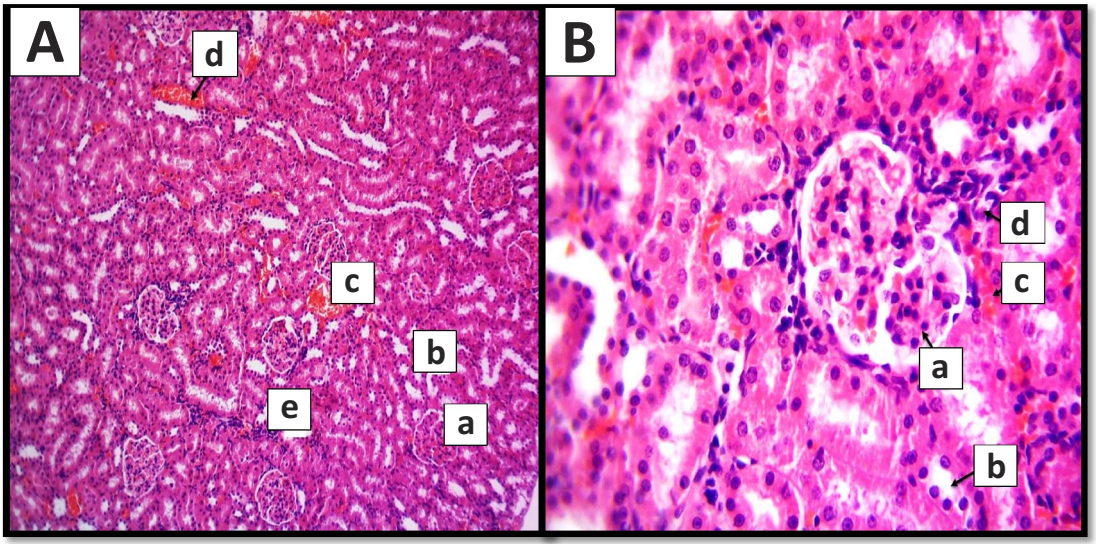
The effect of coadminstration of BC with AMK was evident in improving AMK-induced nephrotoxicity and this was shown in the histological picture of the renal tissues of this group of rats represented by mild dilatation of Bowman's space, mild degeneration of epithelial cells lining renal tubules and hemorrhage (Figures 6) (Table 1).



Figures 6. Photomicrograph of rat kidney of AMK with BC together group shows mild dilatation of Bowman's space (a), mild degeneration of epithelial cells lining renal tubules (b) and hemorrhage (c); A (100X), B (400X), H&E stain.

BC → (BC + AMK) group: (Treated with BC for 9 days then BC and AMK together for 14 days)

The light microscopic investigation of the renal tissue for group pre-treated with BC and then treated with AMK. Also, showed an improvements in decreasing the toxicity caused by AMK including: intact architecture of glomeruli, renal tubules with hemorrhage, congestion of blood vessel and mild infiltration of inflammatory cells (Figures 7) (Table 1).



Figures 7. Photomicrograph of rat kidney of BC treated 9 days then AMK with BC group shows intact architecture of glomeruli (a), renal tubules (b) with hemorrhage (c), congestion of blood vessel (d) and mild infiltration of inflammatory cells (e); A (100X), B (400X), H&E stain.

The scores of the histopathological changes of the kidney

Renal alterations produced by AMK and BC were classified as mild, moderate, or severe. Scores +, ++, and +++ indicate mild, moderate, and severe histopathological changes respectively. The kidney morphological changes are summarized in (Table 1).

Table 1. Histopathological grading of kidney under light microscope.

Histopathological Changes	Control Group	Group B	Group C	Group D	Group E
Atrophy of glomeruli	-	++	-	-	-
Dilation of Bowman's space	-	+++	-	+	-
Vacuolar degeneration of epithelial cell lining renal tubules	-	++	-	-	-
Cloudy cell swelling of epithelial cell lining renal tubules	-	+	-	+	-
Coagulative Necrosis of epithelial cell lining renal tubules	-	++	-	-	-
Inflammation (infiltration of inflammatory cells)	-	++	-	+	+
Hemorrhage	-	+	-	+	+
Congestion	-	+	-	+	+
Hyaline casts	-	++	-	-	-
Renal cysts formation	-	+	-	-	-
Increased fibrous tissue	-	+	-	-	-

Histomorphometric measurements of kidneys among groups of the study

When the five groups' kidney histology results are compared, there are evident disparities in glomerular diameters, Bowman's gap, and the lumen diameters of the proximal and distal convoluted tubes (Table 2).

Table 2. Histomorphometric analysis of kidneys for all groups of the study.

Histomorphometric measurements	Control Group	Group B	Group C	Group D	Group E
Glomerulus diameter (µm)	105.8±3.1 A	95.8±6.2 AC	53.8±6.6 B	88.8±2.5 BC	94.2±3 AC
Bowman's space diameter (µm)	7±0.3 A	8.4±0.7 A	17.7±1.7 B	15.3±1.2 B	8.5±0.7 A
Proximal Convoluted Tubules PCT diameter (µm)	18.9±1.6 A	15.2±1.3 A	35.7±5.6 B	21±1.7 A	16.6±1.2 A
Distal Convoluted Tubules DCT diameter (µm)	25.4±7.3 A	26.9±1.9 A	49.6±9.8 B	39.3±5.9 B	20.7±2.1 A

Data expressed as Mean ± stander error

The different letters in rows means there is a significant difference at $p \leq 0.05$.
PCT (proximal convoluted tubule) lumen, DCT (distal convoluted tubule) lumen.

Discussion

Aminoglycosides are known to cause nephrotoxicity as one of the most important reasons that cause a limitation in their use despite the presence of many benefits of AMGs like their suitable prices and their chemical stability, as well as the fact that they are characterized by giving quick action due to their speed of work against bacteria and the possibility of giving synergistic activity with beta-lactam anti-bacterial medicine (15, 16), all these properties make AMGs essential for severe infections and irreplaceable, which necessarily requires a solution to the problem of nephrotoxicity, which may lead to a worsening of the patient's condition and the possibility of death (17). AMK is an aminoglycoside that has an advantage over the rest of the AMGs in that it can act on bacteria that are resistant to other AMGs. It is currently one of the most described AMGs today and is highly effective against gram-negative bacteria, but as with other AMGs, the induction of nephrotoxicity results in a reduction of its use (18, 19).

According to the experiments that were conducted on humans and animals, the main cause of nephrotoxicity induced by aminoglycosides is oxidative stress (20,21). Oxidative stress occurs when there is an imbalance between oxidants like ROS and antioxidants and is caused by a decrease in antioxidants or when they are depleted as a result of lipid peroxidation, inactivation of antioxidant enzymes, cellular running out of GSH, or destruction of DNA (22). AMK enters the proximal tubule by endocytosis and accumulates in the lysosomes, endoplasmic reticulum, and Golgi body until saturation results in reaching the threshold, then release of AMK into the cytosol and works on mitochondria to cause necrosis (23), this necrosis will decrease the glomerular filtration rate, so the clearance of urea and creatinine will be reduced. That will result in an accumulation or elevation of urea and creatinine levels in the blood (24).

The results showed that the rat group that was treated with AMK showed significant elevations ($p < 0.05$) in the serum urea and creatinine compared to the negative control group, which indicates a reduction of glomerular filtration rate as a result of glomerular tubule damage, and this was confirmed by the histological findings, which showed that the group that was treated with AMK only caused severe damage to the renal tubules. The histomorphometric analysis showed structural changes indicating damage in the renal tubules in the AMK-treated group. All these results give an impression of nephrotoxicity induced by AMK compared to the control group.

These results come in agreement with a study conducted by Abdel-Daim *et al*. (2019) on AMK, which showed that AMK can cause damage to the kidney when it accumulates in the glomerular tubule and this causes an elevation in serum urea and creatinine levels, indicating the presence of renal toxicity evoked by AMK (25). In addition, these findings are consistent with those of research undertaken by Hlail, Faraj, and Wafa S. Abdulredha (2020), which found significant increases in urea and creatinine. They also noted a significant increase in MDA and a significant decrease in GSH as a result of the use of amikacin, confirming the presence of oxidative stress processes. These results suggest that AMK is a cause of nephrotoxicity, and this toxicity could be due to the induction of oxidative stress processes (13). Furthermore, in their study, Mohammed, Abd, and Qasim (2014) showed that the use of AMK can cause nephrotoxicity. There was a significant increase in renal tissue histopathological scores, indicating nephrotoxicity, as well as significant increases in renal function parameters in the AMK-treated group. They also mentioned that this nephrotoxicity was caused by an elevation in ROS, referring to the presence of oxidative stress processes that result in glomerular tubule damage, and this was confirmed by a significant elevation in MDA (26).

In another study conducted by Abdel Fattah and Gaballah (2020), they found a significant elevation in urea and creatinine in the rat group treated with AMK, and this was caused by a reduction in the GFR due to the damage to renal tissues seen in the histological results. These results were associated with the oxidative stress parameters, which showed a significant increase in MDA and a significant reduction in the antioxidant capacity (GSH and catalase). This reinforces the close association between oxidative stress and nephrotoxicity caused by amikacin (27). According to Batoo *et al*. (2018), the use of AMK can result in a significant increase in serum urea, creatinine, and uric acid, which indicates a reduction in GFR indicative of renal toxicity due to increased production of free radicals and lipid peroxidation represented by a significant increase in MDA, and a significant reduction in GSH from the renal cells by unsaturated fatty acid damage of the cell membrane (28).

BC is a member of the CARs family and is responsible for the colors of many fruits and vegetables (29), BC is an excellent source of vitamin A and has numerous therapeutic benefits, such as functioning as an antioxidant, antitumor and being used in photosensitivity therapy (30, 31). The idea of utilizing antioxidants with AMGs to minimize nephrotoxicity developed from this point, and much research has been conducted in this field, with numerous compounds being employed as antioxidants, some of which have been useful in decreasing nephrotoxicity (20). The choice of BC in this investigation comes from several research studies that confirmed that it is efficient in reducing oxidative stress and nephrotoxicity in several trials with various medications.

Our findings showed that in groups treated with AMK concomitantly with BC, the serum urea and creatinine levels were brought down significantly ($p < 0.05$) to near-normal levels, and these findings were confirmed by histological images, which revealed the group that was simultaneously treated with BC and AMK showed nearly normal renal tissue architecture.

According to Mangunsong, Putra, and Taswin (2021), BC extracted from carrots had protective activity against renal toxicity induced by paracetamol. Moreover, BC was able to significantly reduce uric acid significantly ($P<0.05$),

denoting the nephroprotective activity of BC (32). Also, these results are in agreement with a study conducted by Anzel Bahadır *et al*. (2018), where they found that BC can protect cardiac tissues from damage when used in low doses and the histological findings showed improvement of heart tissues as a result of using BC. Furthermore, the study suggests that BC has a potential role in clearing out oxygen species and free radicals, and BC was able to significantly decrease MDA and also significantly elevate antioxidant enzymes (33). Ge *et al*. (2015) demonstrated that BC can reduce free radicals using the ferric reducing antioxidant power (FRAP) method and the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method and that this antioxidant activity of BC can be evoked when BC is placed inside nano micelles (34).

This demonstrated the effect of BC on the ROS and reactive hydroxyl radicals and the protection against oxidative stress and the nephrotoxicity it may cause. It is a highly potent inhibitor of singlet oxygen in nature, and it can also suppress lipid peroxidation (35). The beneficial role of BC varies, and it can protect from nephrotoxicity, hepatotoxicity, and cardiotoxicity by acting on oxidative stress and normalizing their markers. This was demonstrated by many studies conducted on BC (32, 33, 36).

The histological findings of the current study also showed that the rat group coadministered BC with AMK simultaneously showed a normal epithelium of tubules with no degeneration and normal architecture of tubules with mild congestion, which indicates the protective effect of BC against nephrotoxicity induced by AMK, and nearly the same results were given by the group that pretreated with BC and then together with AMK, which indicates that BC exhibits the same protection in the two ways of administration.

In addition to that, the results of the current study showed that the group pretreated with BC and then AMK with BC also showed the protective effect of the BC, which showed a significant decrease ($p < 0.05$) (near the normal) in serum urea and creatinine, and this demonstrates that the pretreatment of BC can give the same protection as that when administered concomitantly. Many studies have been done and pretreated the rats with BC and demonstrated the protective effect of BC by bringing the biochemical markers near normal and also confirmed by the histological results (11, 12).

In a study conducted later by us, the results of that study showed significant elevation of serum MDA level and significant reduction of serum T-AOC and GSH levels in AMK-treated group compared to the negative group, while in group treated with BC together with AMK showed results near normal indicting the protective effect of BC against oxidative stress induced by AMK, and according to this study can cause nephrotoxicity (37).

Although all these studies showed the protective effect of BC against many toxicities induced by oxidative stress and its obvious role in reducing oxidative stress and its toxicities, some studies explained that BC can act in a reversible way and itself can induce oxidative stress and cause toxicity to the heart when given in high doses and even cause cancer in the lung-like in smokers (38-40).

The beneficial outcome of the present study induced by beta carotene should be considered in the protection of renal system from the destructive effects of other chronically used drugs, if any. For example, statins (41, 42), proton-pump inhibitors (43, 44), CNS drugs (45, 46), chemotherapeutic agents (47, 48), drug used for diabetes (49, 50). Moreover, the same positive effects obtained from beta carotene could be provided by other harmless OTC drugs, such as, vitamins (51, 52), or minerals (53, 54), or herbal remedies (55) or xanthine oxidase inhibitors (allopurinol) (56, 57). Nevertheless, claiming potentially positive agents from these drugs need experimental *in vivo* studies on laboratory animal before their clinical application into human, if possible.

Conclusion

The results of the current study lead us to conclude that BC offers significant protection against AMK-induced nephrotoxicity. When given either previously and concomitantly or concomitantly only, BC was able to normalize the elevated renal function tests and histological architecture. Also, results showed the nephrotoxic effect of AMK when given at a high dose and for a long time. However, further pharmacological evidence supporting the role of BC in AMK-induced renal injury is needed to understand the mechanism underlying the alleviation of nephrotoxicity by BC against AMK and its role as a protective agent.

Acknowledgements

The authors are thankful to Pharmacy College and College of Veterinary medicine/University of Mosul for extending the required facilities to carry out this research project.

Conflict of interest

The authors declare no conflict of interest concerned in the present study.

Adherence to Ethical Standards

The study was approved by the Institutional Animal Care and Use Committee in the College of Veterinary Medicine/University of Mosul with approval number (UM.VET.2021.34 in 20.09.2021).

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